



SCIENTIFIC WORKING GROUP ON DNA ANALYSIS METHODS¹

SWGDM Guidelines for Missing Persons Casework

Short Title: *Missing Persons Guidelines*

Effective XXXXXXXX, XX, 202X

Scope

The SWGDAM Guidelines for Missing Person Casework provides guidelines for the processing and analysis of missing persons casework and supersedes the Scientific Working Group on DNA Analysis Methods (SWGDM) Guidelines for Missing Persons Casework (2014). DNA profiles developed from unidentified human remains and unidentified living persons may be compared directly to references from a family at the request of the investigating agency when the facts of the case lead to a preliminary hypothesis of identity. Alternatively, the DNA profiles from remains without a presumed identity can be compared to a DNA database of missing persons and family reference samples, such as the Combined DNA Index System (CODIS).

¹The Scientific Working Group on DNA Analysis (SWGDM; see SWGDM.org) is comprised of forensic science practitioners and other experts who represent government laboratories within the U.S and Canada, as well as intra- and international professional groups and academia. SWGDAM recommends to the FBI Director revisions to the *Quality Assurance Standards for Forensic DNA Testing Laboratories* and the *Quality Assurance Standards for DNA Databasing Laboratories (QAS)*. SWGDAM provides a forum for its members and invited guests to discuss research, technologies, techniques, and training; and conduct or recommend studies to develop, test, and validate methods for use by forensic laboratories. SWGDAM's Guidelines and Recommendations represent best practices within the discipline. The term "should" is used herein to indicate good practices identified by SWGDAM. "Shall" distinguishes mandatory elements, which may be specified in the *Quality Assurance Standards for Forensic DNA Testing Laboratories* and/or *Quality Assurance Standards for DNA Databasing Laboratories*.

**SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023**

Table of Contents

1. Introduction.....	2
2. DNA Recovery From Human Remains.....	3
3. Reference Samples.....	7
4. Metadata.....	10
5. Targeted Comparisons.....	11
6. CODIS Comparisons.....	12
7. Resolving Associations.....	16
8. Reporting Associations.....	18
9. Investigative Genetic Genealogy (IGG).....	20
10. Resources.....	21
11. References.....	24
Appendix A.....	27
Appendix B.....	35

Key Concepts:

- ❖ This document outlines important considerations for skeletal samples which may not be routine evidence for most laboratories.
- ❖ Examples of family trees are provided to assist laboratories in determining from whom samples should be collected in a missing persons case.
- ❖ The types of CODIS searches performed for missing persons casework are specified as well as considerations in resolving and reporting associations.
- ❖ Additional resources include laboratories that have developed missing person programs as well as other organizations that are dedicated to solving missing person cases.

1. Introduction

The intent of this document is to provide forensic laboratories with foundational information for conducting missing persons casework in the United States that may involve the use of the Combined DNA Index System (CODIS) (see Section 6). It is the responsibility of each organization to develop protocols, policies, and procedures consistent with those of its

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

criminal casework component as well as the local medico-legal authority.

The comparison of DNA profiles from unidentified human remains (UHR) to samples attributed to the missing person or biological relatives of a missing person can provide a powerful tool for the medico-legal authority to aid in the identification process. The identification of individuals and human remains can be accomplished by several methods. Recognition by the next of kin and the comparison of ante-mortem and post-mortem biometric data such as dental X-rays and fingerprints are common methods of identification. DNA analysis is available when these methods are not successful or additional information is needed.

DNA profiles developed from human remains may be compared directly to references from a family at the request of the investigating agency when the facts of the case lead to a preliminary hypothesis of identity. Alternatively, the DNA profiles from remains without a presumed identity can be compared to a DNA database of missing persons and family reference samples, such as CODIS. CODIS contains indices for unidentified human remains, direct missing person reference samples and voluntarily contributed family reference samples that are routinely searched to assist in the identification of the missing.

Familial searching and investigative genetic genealogy (IGG) are two additional techniques that have been used to develop leads for finding relatives of unidentified human remains. The use of these techniques for missing persons cases should be guided by the policies and procedures outlined by the state or local jurisdiction.

The guidance outlined in this document may also apply to casework involving unidentified living persons.

2. DNA Recovery from Human Remains

Prior to conducting DNA analysis, a multidisciplinary approach involving experts from other disciplines in the identification process should be considered. The assistance of a forensic anthropologist can be useful in the recovery of evidence and skeletal remains. For many cases, the forensic anthropological examination can determine if a specimen is a bone and whether a

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

bone is human or non-human. Additionally, an anthropologist can detect and document tool marks as well as ante-mortem and post-mortem skeletal damage. This examination may also determine if the recovered remains represent the comingling of more than one decedent. An anthropological examination can also provide an assessment of sex, age at death, ancestry, stature, and time since death, depending on which remains are recovered and the condition of them. However, it is important to note that discrepancies can occur between an anthropological determination of sex and the genetic determination of sex (e.g., amelogenin genotype or DYS391). Prior to processing teeth, a forensic odontologist should perform a dental examination to chart the types of teeth recovered, the position and condition of the teeth, and any dental restorations. Post-mortem dental X-rays should also be taken. Forensic anthropology and odontology examinations should be performed prior to DNA analysis. If it is not feasible to have these examinations performed prior to DNA analysis, consultation with an anthropologist or odontologist should be considered to ensure that the samples used for DNA analysis will not affect potential examinations at a later date should they be determined to be needed.

For the purposes of this document, an unidentified human remain(s) is a deceased body, including body parts or tissues, whose identity is not known. Unidentified human remains submitted to laboratories can vary from a bloodstain to skeletal samples. The success of DNA analysis generally depends on the length of time since death, the type of biological sample recovered and the degree of decomposition or degradation. Blood is generally the best choice, since it contains a relatively high amount of intact DNA. Muscle tissue, nails, ocular swabs and head hairs are useful sources of DNA from decomposed remains. Skeletal remains are processed when other samples are not available or when preferred samples do not yield sufficient DNA. Traditionally, long bones and unrestored teeth are the most commonly submitted samples for identification purposes. However, other smaller bones may be a better source of DNA (petrous bone, patella, or foot bones such as the talus, cuboid or cuneiform). (Mundorff and Davoren, 2014).

An unidentified human reference sample is a buccal swab or blood draw obtained directly

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

from an unidentified deceased person before there has been indicia of decay of the body. Such samples may be processed as a reference sample under the Federal Bureau of Investigation's (FBI) *Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories*. It is recommended that unidentified human reference sample(s), as defined above and processed as a reference sample under the QAS, be allowed at NDIS in the Unidentified Human Remains Index. The National DNA Index System (NDIS) is the national level of CODIS, containing the DNA profiles contributed by federal, state, and local participating forensic laboratories (see Section 6).

Bones should be properly decontaminated via sanding, washing in a detergent solution, or other validated procedure, prior to DNA analysis. After surface cleaning, a portion of the bone is excised. Generally, this entails the use of a cutting blade attached to a rotary tool. A section or cutting of the skeletal sample to be used in the extraction process is generally powdered or fragmented using a freezer mill, blender, or drill. The sanding and cutting of bones presents both a safety concern as well as a contamination risk for the laboratory. Therefore, these procedures should be performed in a hood, preferably under negative air pressure. Proper personal protective equipment should be used to minimize exposure. Care should be exercised to avoid cutting completely through a bone or taking a section from an anatomically and/or forensically significant location.

In addition to cutting a sample for extraction, it is good practice to cut and retain a portion of the remains for future DNA analysis. Retained samples are maintained in the event of advances in techniques that increase the possibility of obtaining a complete genetic profile (such as improvements in DNA isolation and amplification) and the adoption of additional DNA markers. The submitting agency should be notified that a portion of the sample is being retained.

The extraction of DNA from skeletal remains is similar to the DNA extraction from other types of forensic samples. The goal is to isolate the DNA from the sample and purify it to obtain a suitable DNA template for the typing of polymorphic autosomal (e.g., STR, Single Nucleotide Polymorphism (SNP)) and lineage (e.g., Y-STR, mtDNA) markers. Various

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

methods of DNA extraction can be used to provide a suitable DNA extract from the bone sample including both organic and inorganic methods. Each laboratory should evaluate the suitability of its current methodologies and standard operating procedures for use with skeletal remains. Laboratories interested in adopting procedures for processing skeletal samples for DNA analysis can obtain more information from the referenced papers. They can also request a copy of the DNA extraction procedures for skeletal samples from laboratories currently conducting analysis of unidentified human remains (see Section 10).

Unidentified human remains are often recovered and analyzed months or years after death and are routinely exposed to adverse environmental conditions. These conditions can negatively impact the success of DNA typing through the degradation of DNA and the introduction of PCR inhibitors. The extent of degradation and inhibition depends on temperature, humidity, microbial growth, and the length of time in the environment. Laboratories may use DNA extraction methods with silica purification techniques to overcome PCR inhibition. If silica purification is unsuccessful in the removing of inhibitors, dilution of DNA extracts or modified PCR reaction mixes can be utilized (Alaeddini, 2012). For samples with highly degraded DNA, it may be necessary to type the sample with testing systems amenable to small fragment sizes, such as mini-STR kits and mini amplicon assays for mtDNA. SNPs and/or Next Generation Sequencing (NGS) may also be useful for typing samples that are highly degraded.

In accordance with the NDIS Operational Procedures Manual (<https://le.fbi.gov/file-repository/ndis-operational-procedures-manual-version-12-070123.pdf/view>), DNA records developed from unidentified human remains and other single source samples from missing person investigations or mass disasters using Low Template or Low Copy DNA Analysis validated in accordance with the QAS and SWGDAM Guidelines for STR Enhanced Detection Methods (available at www.SWGDAM.org) may be submitted to NDIS.

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

3. Reference Samples

Reference samples are collected either from the relatives of a missing person or from samples attributed directly to the missing person. These reference samples are subsequently compared to unidentified human remains to facilitate identifications. Family reference samples usually consist of buccal samples or dried blood stains. For inclusion in the National DNA Index System (NDIS), the collection requirements in the NDIS Operational Procedures Manual must be followed. In other scenarios which do not need NDIS entry (e.g., mass disaster investigations), other collection policies may apply. The donor's identity and relationship to the missing person should be verified and documented. Family references should also be submitted with a consent form signed by the donor (or the donor's legal guardian). The consent form should indicate that the sample was donated voluntarily for the purpose of identification of a missing person. It should also indicate the extent to which the profile may be searched in a DNA database. Reference samples that are not submitted by law enforcement agencies with the appropriate documentation may not be suitable for entry into CODIS.

Ideally, reference samples for missing persons cases should be obtained from at least two biological relatives of the missing individual (Figure 1). However, a single relative can still provide valuable genetic information, especially if lineage marker testing is employed. The most informative samples are from individuals closely related to the missing person such as parents, children, and siblings, also known as first degree relatives. Parents and children share exactly 50 percent of their alleles (barring mutation) and they have the additional advantage of permitting exclusions to be made, as each locus must have at least one shared allele. On average siblings share 50 percent of their DNA; however, at each autosomal STR locus siblings can share no alleles, one allele or two alleles which leads to difficulty in making definitive exclusions. In the absence of parents or children, it is very useful to have multiple siblings as references. Lineage marker testing may also be helpful in the absence of reference samples from parents or children.

The next most informative relatives are second degree relatives who share on average 25 percent of their genetic makeup and include aunts, uncles, half-siblings, and grandparents.

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

Third degree relatives share on average 12.5 percent of their genetic makeup and include an individual's great-grandparents, great-grandchildren, great-uncles/aunts, and first cousins. If the only relatives available are distant relatives (e.g., second or third degree), autosomal STR markers may not be as useful; however, lineage markers such as mtDNA (maternal lineage) or Y-STRs (paternal lineage for a missing male) may bridge the gap for useful genetic comparisons (see Figures 2A, 2B and 3). In addition, SNP analysis may be useful for these distant relatives.

For a missing person case where a biological child is submitted as a reference sample, it is also useful to submit a reference sample from the other biological parent of the child. The resulting profiles can be used to create a pedigree tree. If law enforcement is approached by multiple individuals wishing to donate samples, they should be encouraged to collect a reference sample from each person. The laboratory can then determine which samples will be best suited for analysis and pedigree construction. The importance of verifying the donor's biological relationship to the missing person cannot be overstated. Improperly stated or documented relationships may lead to missed identification opportunities.

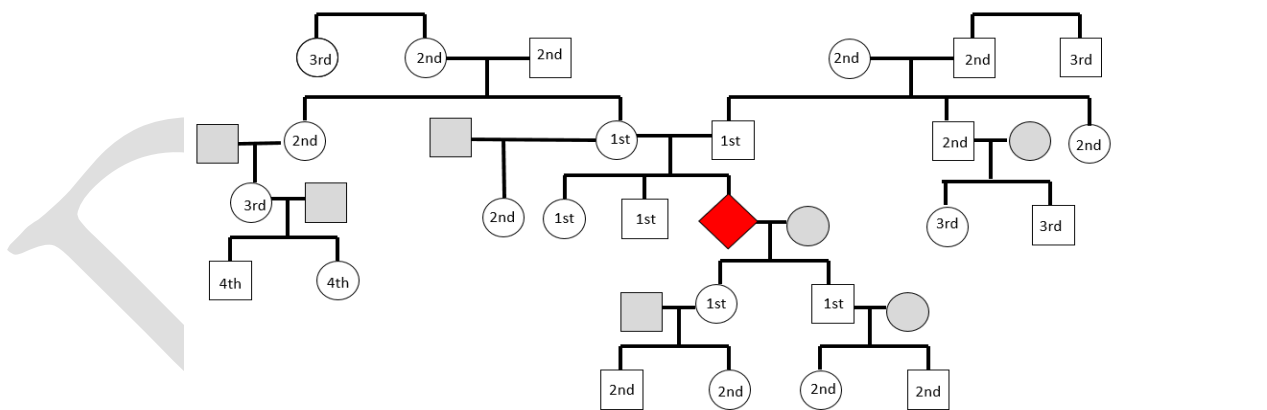


Figure 1. An example pedigree displaying 1st, 2nd, 3rd and 4th degree relatives of the missing person (red diamond). Individuals shaded in grey are genetically unrelated to the missing person (including spouse).

(Key: Missing Person-Diamond, Male XY-Square, Female XX-Circle)

1st degree relatives: parents, full siblings and children

2nd degree relatives: aunts, uncles, grandparents, grandchildren, nieces, nephews, half-siblings

3rd degree relatives: great-grandparents, great-aunt, great-uncle, first cousins

4th degree relatives: 1st cousins once removed (children of 1st cousin)

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

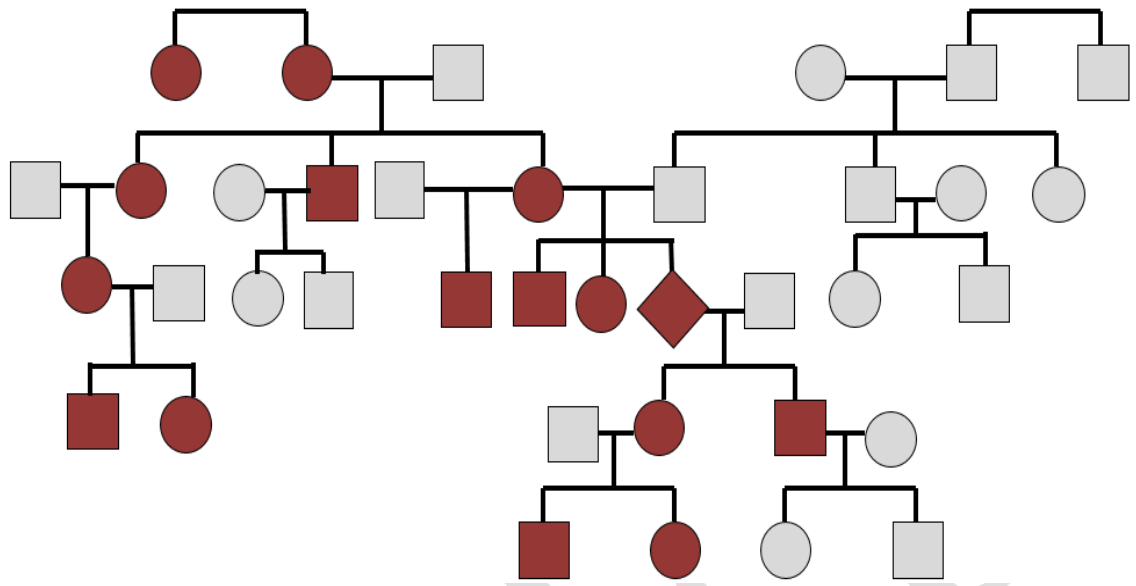


Figure 2A. Pedigree depicting pool of reference individuals sharing the same mitochondrial DNA haplotype for a missing female individual (barring mutation) in maroon. Grey shaded individuals are maternally unrelated to the missing person. (Key: Missing Person-Diamond, Male XY-Square, Female XX-Circle)

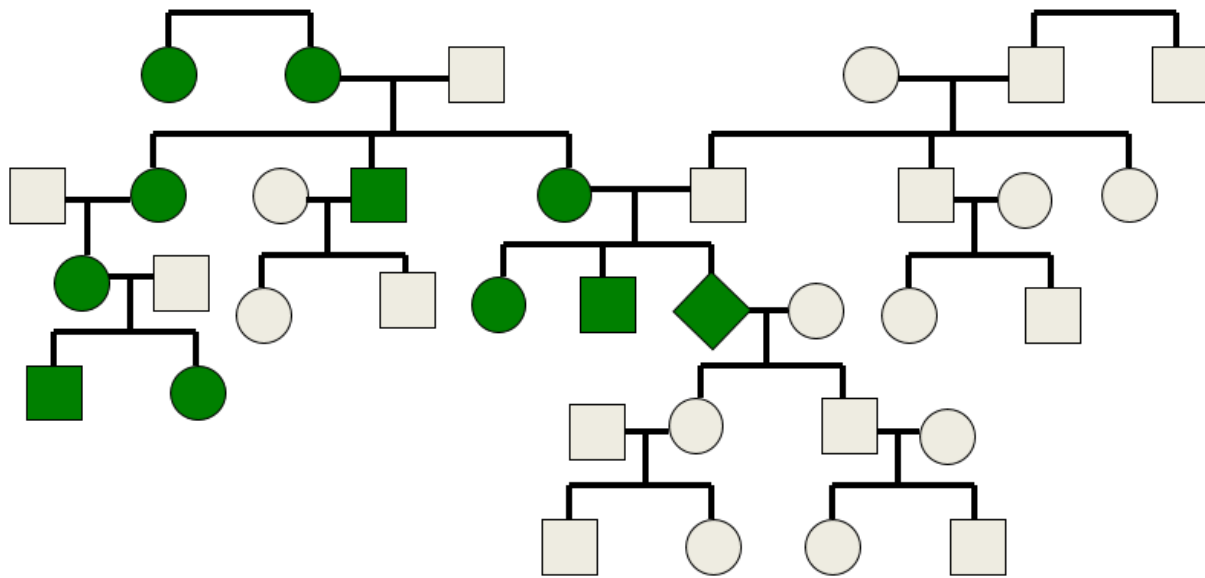


Figure 2B. Pedigree depicting pool of reference individuals sharing the same mitochondrial DNA haplotype for a missing male individual (barring mutation) in green. Grey shaded individuals are maternally unrelated to the missing person. (Key: Missing Person-Diamond, Male XY-Square, Female XX-Circle)

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

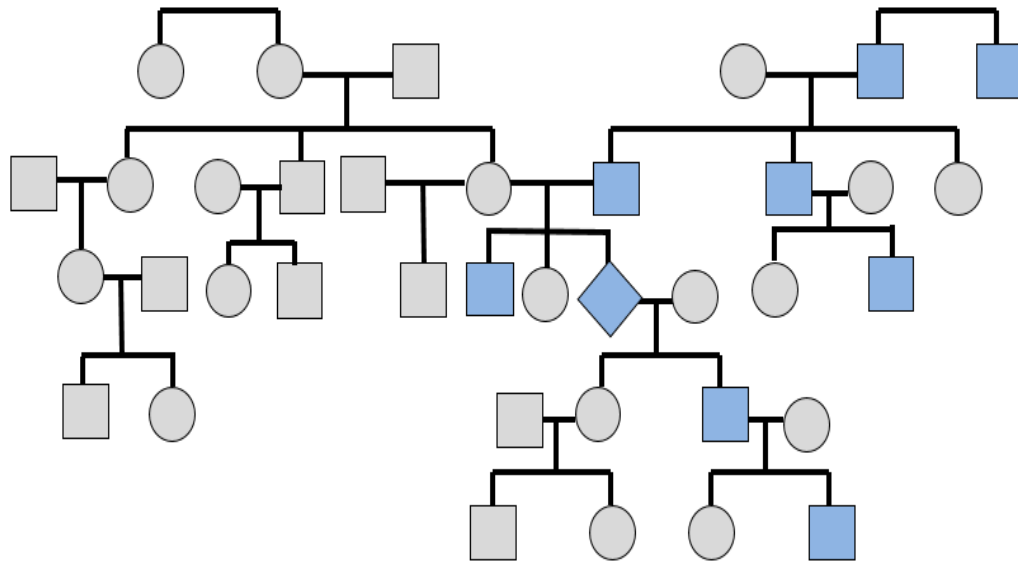


Figure 3. Pedigree depicting pool of reference individuals sharing the same Y-STR haplotype for a missing male individual (barring mutation) in light blue. Grey shaded individuals are paternally unrelated to the missing person.
(Key: Missing Person-Diamond, Male XY-Square, Female XX-Circle)

Direct reference samples from missing persons are categorized either as a deduced missing person sample or as a missing person sample. A deduced missing person sample is an item that is believed to be attributed to the individual such as a toothbrush, razor, or article of clothing. The deduced missing person DNA typing results should be compared to those of known family reference samples, when available, to ensure that the results obtained are consistent with belonging to the missing person. In contrast, a missing person sample can be independently verified through other documentation as coming directly from the missing person (e.g., a clinical biopsy sample or newborn screening specimen). For a thorough discussion of various sources of DNA from personal effects, see Prinz *et al.* (2007).

4. Metadata

Metadata is non-DNA information that is used in conjunction with DNA analysis to help resolve possible associations between missing and unidentified persons (see Table 1).

**SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023**

Metadata for the remains is obtained from reports produced by other forensic disciplines such as anthropology, odontology, and pathology as well as the law enforcement agency’s report. For a missing person, metadata is gathered from police reports, investigators, and family members. Information on the date of last contact, the last known geographic location, the date of birth, and physical anomalies are just a few examples. After evaluating the genetic data of a potential association, metadata should be compared to validate or refute relatedness provided by the DNA results. It is important to note that metadata can be incorrect, including anthropological determination of sex, ranges of age and height estimate, and ancestry. All laboratories and investigating agencies involved in evaluating possible associations should review the sources and limitations of the metadata before any final conclusions are reached.

Table 1: Metadata categories used to evaluate potential associations

Unidentified Human Remains (UHR)	Missing Person
Sex	Sex
Ethnic Group	Ethnic Group
Age Range	Date of Birth
Height	Height
Dental Records Available	Dental Records Available
Specimen Origin (whole/partial remains)	
Physical Anomalies	Physical Anomalies
Scars/Marks/Tattoos	Scars/Marks/Tattoos
Date of Recovery	Date of Last Contact
Geographic Location of Recovery	Geographic Location of Last Contact
City	City
State	State
Location	Location

5. Targeted Comparisons

Laboratories often receive requests to conduct specific comparisons when unidentified human remains have been linked to a specific missing person through investigations conducted by law

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

enforcement and/or medico-legal authorities. Typically, the laboratory would receive a sample from a set of unidentified human remains as well as samples collected from the relatives of a missing person or a direct reference sample from the missing person. The samples would come with a specific request to compare the DNA results. This could result in support for identity, relatedness, or an exclusion. Depending on the amount of genetic data obtained from the remains and/or the relatedness of available references, likelihood ratios (LRs) may vary from strong support to uninformative. The laboratory's policy should address how the comparison is reported in such instances. In situations where the statistical calculations do not sufficiently support the proposed relationship, the laboratory can still enter the eligible samples into the CODIS database. If an identification is made to a partial set of unidentified human remains, the decedent's DNA profile should be placed into the CODIS database in the event that additional remains are recovered at a later time.

It is important to note that DNA analysis is a tool utilized to assist the medical examiner/coroner in determining the identification of an individual. The laboratory does not make identifications.

6. CODIS Comparisons

CODIS is the acronym used for the FBI's program of support for criminal justice DNA databases as well as the software used to run these databases. NDIS is considered one part of CODIS, the national level, containing the DNA profiles contributed by federal, state, and local participating forensic laboratories. For the best chance of making an association, it is important to have an NDIS-eligible profile (see NDIS Operational Procedures Manual).

The DNA Identification Act of 1994 (34 U.S.C. §12592) authorized the establishment of this National DNA Index. The DNA Act specifies the categories of data that may be maintained in NDIS (convicted offenders, arrestees, legal, detainees, forensic (casework), unidentified human remains, missing persons and relatives of missing persons) as well as requirements for participating laboratories related to quality assurance, privacy, and expungement.

For missing persons, relatives of missing persons, and unidentified human remains samples,

SWGDM Missing Persons Casework Guidelines **Draft for SWGDAM Comment 12052023**

relevant additional DNA technologies other than autosomal STR typing (such as mtDNA, Y-STR, X-STRs or SNPs) should always be considered. The lack of an additional typing system will not render a sample ineligible for entry into CODIS, but analysis using an additional appropriate system is recommended to ensure the most robust searching possible.

For autosomal results at a given locus, a single allele should not be entered into CODIS if there is a high potential for sister allele drop-out based on validation. This locus should be left blank since it may result in a false exclusion.

A pedigree utilizing the relatives of the missing person should be created in the CODIS software. A pedigree is a graphical representation that describes the relationships among the missing person and his/her relatives. The more informative pedigrees have at least one first degree relative of the missing person and both STR and lineage marker data. Note that multiple pedigrees for a missing person can be made in CODIS if proposed relationships are uncertain.

6.1 Identity Searches

Unidentified human remains profiles can be compared in a pairwise manner to profiles in various CODIS indexes in an attempt to discover a direct match to the source of the unidentified profile, or to discover an association to profiles of closely related family members of the missing person (i.e., partial match). A pairwise search can provide very strong evidence for identification if the unidentified remains come from an individual whose profile had previously been entered into a searchable index.

6.2 Low Stringency Identity Searches (requires second DNA technology)

Using CODIS, close relatives can be found with a low stringency search to find profiles sharing at least one allele per STR locus and consistent results with at least one lineage marker. Under certain circumstances, such as when there may be inconsistencies in the stated relationships within the pedigree, pairwise comparisons can indicate a relationship association that is erroneously excluded by pedigree searches.

SWGDM Missing Persons Casework Guidelines Draft for SWGDAM Comment 12052023

6.3 Pedigree Tree Searches

Once a pedigree tree is created from typed family members, a pedigree tree search compares the DNA profiles from an unidentified person to those of the typed family members in a single search. This allows for a more robust search than the pairwise comparisons mentioned above. After the search is performed, a rank may be returned involving a pedigree and an unidentified human remains specimen. For each pedigree, a ranked list of unidentified human remains will be generated using a (LR) (see Section 6.4 below). The same unidentified human remains may be returned as a rank to multiple pedigree trees among different laboratories; all should be fully reviewed regardless of the LR given to the rank. The true biological relationship may not be represented by the rank with the highest LR, since that statistic is dependent on the number and types of relatives present in a particular pedigree. In other words, a weak pedigree, consisting of more distant relatives, may return a lower LR than a stronger pedigree tree, with first degree relatives. The weak pedigree may contain the true biological relatives of the unidentified person while the stronger pedigree may have been returned as a fortuitous rank. Evaluation of metadata should aid in determining the correct association.

6.4 Statistical Relevance

CODIS uses LRs to evaluate and rank candidate associations during a pedigree search. Generally speaking, an LR is the ratio of the following two conditional probabilities:

H₁: The probability of observing the genetic profiles from the unidentified remains sample and the family reference samples if the remains represent the missing individual in the established pedigree of the family references;

H₂: The probability of observing the genetic profiles from the unidentified remains sample and the family reference samples if the

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

remains represent an individual unrelated to the established pedigree of the family references.

CODIS calculates a separate LR for each genetic typing system. The Joint Pedigree Likelihood Ratio (JPLR) is computed for autosomal STRs. A Y-STR LR and a mtDNA LR are computed for each of those systems. The available LR values are multiplied together to generate the Combined Likelihood Ratio (CLR). For a pedigree tree to be returned in a rank, the CLR must exceed a threshold designed to limit the number of false associations returned during a search. The intention is to strike a balance between limiting the number of false associations returned against the potential of not returning the true association if the statistic falls below the threshold.

It is important to stress that the LR values calculated by CODIS are only for the ranking of pedigree tree associations, and NOT for statistical reporting. This is because CODIS combines several population groups to generate allele frequencies, whereas reported LR values are typically calculated separately by population group. PopStats or another freely available statistical tool (e.g., KIn CALc or Familias) should be used for the calculation of LR values for reporting purposes.

If reporting combined statistics for autosomal STRs, mtDNA and/or Y-STR results, the laboratory issuing the report should verify that peer-reviewed publications have demonstrated genetic independence between the marker systems. In other words, the literature must indicate that the populations(s) under consideration do not show evidence of linkage disequilibrium among the marker systems that the laboratory wants to combine. If linkage disequilibrium was observed among the marker systems in a population, this must be accounted for in the combined marker set statistics.

Similarly, even when linkage disequilibrium is not detected in the population, physical linkage of autosomal STR may need to be accounted for in certain pedigrees when loci are present on the same chromosome and the recombination factor is below 0.5. For example, linkage should be considered when combining vWA and D12S391 where the

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

recombination factor is estimated to be between 0.089 and 0.117. (ISFG, O’Conner, 2011). Simple paternity cases involve a single genetic transmission event so they do not need corrections for physical linkage between loci as long as the loci are in linkage equilibrium at a population level (ISFG, O’Conner, 2011). Pedigrees that contain any other relationship (sibling, half-sibling, grandparent-grandchild, cousins, etc.) will be impacted by linkage if closely linked loci are used.

It is imperative for the laboratory to develop policies and procedures as well as train staff on the use of statistical tools used for kinship analysis before profile entry into CODIS.

7. Resolving Associations

Following a targeted comparison or a database search, additional genetic and non-genetic data may be useful in evaluating the validity of the proposed relationship between unidentified human remains and reference samples. Depending on the particular family members present in a pedigree tree, the amount of genetic data, and the metadata, it may not be possible to exclude a biological relationship between a pedigree tree and an unidentified human remains sample, whether there is a true biological relationship or not.

7.1 Statistical Relevance

When a rank is evaluated, the CODIS core loci and the available lineage markers that are part of the rank are evaluated first. Many times, additional STR data are obtained during the analysis process and are available for comparison purposes. Those additional loci should be reviewed to determine if they can be used to exclude the unidentified remains from the pedigree association. If appropriate population databases are available, those loci can also be used in subsequent LR calculations. The necessity for additional DNA information should also be evaluated at this time. For example, if an unidentified remains sample was only typed using autosomal STRs and was potentially ranked to a pedigree containing two full siblings, both with autosomal STRs and one that also had mtDNA, then it may also be informative to develop a

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

mtDNA profile for the unidentified remains to use in the comparison.

7.2 Review Metadata

The metadata should be evaluated secondarily to the DNA typing results and used in order to potentially include or exclude the rank. Metadata for both the missing person and the unidentified human remains should be evaluated to assess consistency. For example, a date of last contact for the missing person after the date the body was found could exclude the association. Another example would be a different genetic sex for the unidentified remains and the missing person. Vastly differing ages between the missing person and the unidentified remains may also be a reason to exclude an association. However, it is important to note that metadata can be incorrect, particularly where date ranges, age estimates and ancestries are involved.

7.3 Additional Considerations

If an exclusion cannot be made solely on the DNA results and the metadata provided, each laboratory is responsible for communicating with the other laboratory. Each laboratory is also responsible for contacting their respective submitting agencies in order to obtain additional information and/or reference samples that could help in evaluating an association.

Additional family member reference samples may be necessary in order to build a stronger pedigree. Another sample from the unidentified remains may also be required in order to obtain additional DNA data, such as mini-STRs, Y-STRs, mtDNA, X-STRs or SNPs.

In mass disaster scenarios (e.g., wildfire or a plane crash) it is imperative that there is communication between all agencies involved, including temporary laboratories set up to perform analysis. Additionally, identification of victims via DNA may require specific statistical issues that need to be addressed (Brenner, C.H and Weir, 2003; Brenner 2005; Bailey-Wilson J.E., 2006; Sozer, A. et. al, 2010)

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

8. Reporting Associations

Typically, the laboratory that entered the profile for the unidentified human remains will take the lead in writing the report resulting from a CODIS association. Because CODIS allows for mutational events, the reporting laboratory should be able to accommodate mutations in kinship analysis calculations. The laboratory should use reporting language that accurately conveys the strength of the association to the submitting agency.

8.1 Statistical Evaluation

Missing persons casework encompasses a myriad of possible scenarios requiring appropriate statistical interpretation for an association or competing associations. Simple cases may require only a standard parentage or a reverse parentage calculation, in which the missing person (represented by the unidentified remains profile) is a parent or child, respectively, of a trio. These types of calculations can be performed using the existing tools in PopStats or other similar software packages. However, many cases present a more complex pedigree of known samples provided by family members of the missing person. Comparison of the genetic data from an unidentified human remains sample to a pedigree of family reference samples is the appropriate and most powerful statistical approach for evaluating a database association. Statistical evaluation of pedigree data is most commonly done utilizing modifications and amendments to the algorithm described by Elston and Stewart (1971). Examples of such calculations are discussed in Appendix A.

It is imperative that the laboratory examines the pedigree (for example using a freely available statistical tool like KIn CALc or Familias) for consistency prior to its use in comparison to the unidentified remains, especially if additional reference samples were added following an association. Different hypotheses may be considered during the

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

statistical analysis to determine the best fit of the unidentified remains data to a reference pedigree or alternate pedigrees. Instances involving mutational events could occur with STRs. The mutation rate of a single autosomal STR locus is low (approximately 10^{-3} per generation). However, the probability of observing a mutation increases when multiple family members, especially siblings, are included in the pedigree or a large number of loci are typed. These inconsistencies must be evaluated in terms of alternate hypotheses surrounding the observation of the particular set of genetic data (Brenner 2004) and may result in the consideration of multiple pedigree trees.

Another possibility is that the defined relationships in the pedigree are incorrect as reported (e.g., a presumed full sibling may actually be a half sibling). Alternate relationships in the pedigree should be investigated statistically, and if possible, through clarification by the submitting agency of the reference samples.

Inconsistencies will result in a reduction of the statistical power inherent in the genetic data. Laboratories should have policies in place for the reporting of relationships that are different than those stated by the family (e.g., non-paternity; Parker et al., 2012).

The report must clearly state the hypotheses examined that yielded the reported LR. Laboratories may choose to adopt the convention of the relationship testing community (AABB, 2011) and report the LR based on the relevant population group (e.g., that of the pedigree members, hence the missing person). Alternatively, laboratories may report statistics in the manner used for criminal casework as determined by their laboratory protocol (see Appendix A).

8.2 Developing Reporting Policies

It is important for every laboratory to develop policies for the release of personally identifiable information regarding an association, such as the names and the biological relationships of the tested individuals. It may be the laboratory policy to only report the findings to the agency that submitted the remains sample, where other laboratories may also send a copy of the report to

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

the laboratory(ies) that submitted the family reference samples. Laboratories should have written policies in place regarding information being released to entities other than the original submitting agency. If multiple laboratories are involved in an association, only one laboratory should generate a statistical report for the association.

9. Investigative Genetic Genealogy (IGG)

Since 2018, IGG has been successfully used to identify previously unidentified human remains (UHRs). After genotyping with STRs, a SNP profile developed from the UHR sample may be searched in one or more publicly available personal genetic genealogy DNA databases that allow access for searching of UHRs (e.g., GEDmatch, FamilyTreeDNA). The file can then be compared against the SNP files of individuals who voluntarily submitted their biological samples or entered their genetic SNP files into these databases. Computer algorithms are used to evaluate potential familial relationships between the UHRs and the individuals in the database. A list of potential genetic relatives is then generated based on shared segments of DNA.

Laboratories have experienced difficulty producing a SNP file with SNP array analysis from skeletonized remains that can be successfully searched in online genetic genealogy databases. However, it should be noted that whole genome sequencing (WGS) and targeted sequencing have been successfully used as alternatives to generate SNP files for genetic genealogy searches of UHR's containing degraded DNA and samples with high ratios of microbial DNA (Loreille et al, 2011; Tillmar, 2020 and Kling et.al. 2021).

In 2019, the U. S. Department of Justice issued its *Interim Policy on Forensic Genetic Genealogical DNA Analysis and Searching* (available at <https://www.justice.gov/olp/page/file/1204386/download>). The interim policy should be consulted by entities considering the use of IGG for investigations involving missing persons and unidentified human remains involving a suspected homicide victim. The interim policy addresses the genetic privacy practices that DOJ agencies and other entities that receive DOJ assistance and funding are required to follow. Additionally, the interim policy requires that investigators first

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

obtain a CODIS STR profile from evidence or UHRs and that the STR profile be searched in CODIS before attempting SNP analysis.

Currently, SNP and WGS results have not yet been widely used as a means of human identification in U.S. courts. For these reasons, after using SNPs to identify potential relatives in online genetic genealogy databases, it is recommended to conduct STR typing on the relatives for comparison to the UHR. Mitochondrial DNA and/or Y-STR typing can also be used when complete STR profiles have not been obtained for the UHR or, as appropriate, depending upon the available relatives.

10. Resources

10.1 DNA Resources

Laboratories that have developed missing persons programs are available to assist laboratories in establishing similar programs. Technical procedures, reporting criteria, consent forms and submission guidelines can be obtained from each of the following laboratories:

Armed Forces Medical Examiner System's Armed Forces DNA Identification Laboratory (AFMES-AFDIL);

<https://health.mil/Military-Health-Topics/Combat-Support/Armed-Forces-Medical-Examiner-System/DoD-DNA-Registry/DNA-Identification-Laboratory>

California Department of Justice, Bureau of Forensic Services, Jan Bashinski DNALaboratory

<https://oag.ca.gov/missing/mups>

Federal Bureau of Investigation

<https://le.fbi.gov/science-and-lab-resources/biometrics-and-fingerprints/>

Minnesota Bureau of Criminal Apprehension

<https://dps.mn.gov/divisions/bca/bca-divisions/forensic-science>

New York City Office of Chief Medical Examiner

<https://www1.nyc.gov/site/ocme/services/department-of-forensic-biology.page>

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

Royal Canadian Mounted Police

<https://www.rcmp-grc.gc.ca/en/national-missing-persons-dna-program>

University of North Texas Center for Human Identification

[Homepage - Center for Human Identification \(unthsc.edu\)](http://unthsc.edu)

10.2 Non-DNA Resources

All states have missing persons clearinghouses that can be of assistance in obtaining information such as metadata or obtaining additional family reference samples to support an association (www.missingkids.org/clearinghouses). In addition, several organizations are involved in the efforts to match missing persons and unidentified human remains using non-DNA information and are listed below (in alphabetical order). Many of these websites will indicate whether DNA is available and the laboratory contact information, but only CODIS conducts national DNA searches between missing and unidentified persons.

NamUs (<https://namus.nij.ojp.gov/>)

The National Missing and Unidentified Persons System (NamUs) is an on-line repository of physical information for both missing and unidentified persons that allows for cross-matching and searching. It is publicly accessible and searchable, with restricted access to law enforcement and coroners/medical examiners for case information entry. The availability, location and type (STR, Y-STR or mtDNA) of DNA data can be noted for each case.

NCIC

The National Crime Information Center (NCIC) is a repository of information restricted to law enforcement agencies. It includes information on both people and property, involving several categories of crime such as wanted persons and stolen vehicles. There are also records of missing persons and unidentified persons. These records may include metadata such as the date of birth, sex, race, and dental records for a missing person. For unidentified persons, the

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

records may include the estimated age, sex, race, estimated weight and height, date found, personal effects, and dental records. There is also information on whether DNA results are available and which laboratory processed the samples.

NCMEC (www.missingkids.org)

The National Center for Missing and Exploited Children (NCMEC) is a non-profit entity chartered by Congress. NCMEC provides assistance to families and law enforcement in cases of missing children.

ViCAP

The FBI's Violent Criminal Apprehension Program (ViCAP) was developed to track violent crime data. In addition to homicides and sexual assaults, ViCAP also contains information related to missing and unidentified persons, including photographs and other biographical data.

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

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SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

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SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

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SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

Appendix A. Pedigree Statistical Evaluation Example

Unlike routine identity comparisons or single reference relationship analysis (e.g., parent-offspring, sibling-sibling, etc.), evaluation of more extensive pedigrees to unidentified human remains associations are complex. Ultimately, the working hypotheses of these evaluations are: H_1 : The probability of observing the genetic profiles from the unidentified remains sample and the family reference samples if the unidentified remains represent the missing individual in the established pedigree of the family references, or H_2 : The probability of observing the genetic profiles from the unidentified remains sample and the family reference samples if the unidentified remains represent an individual unrelated to the established pedigree of the family references. It is important to note that in certain circumstances, the unidentified remains may actually represent a different missing member of the same family. These additional alternate hypotheses should be evaluated if the case scenario warrants.

Each of the hypotheses can be represented by a conditional probability that takes into account the following three items: (1) the particular relationships among the members of the pedigree and the unidentified individual; (2) the probability associated with allele sharing given the degree of relationship among the members of the pedigree and the unidentified individual; and (3) the frequencies of the obligate alleles/genotypes that contribute to the structure of the pedigree.

Although the actual process of calculation will not be discussed here, the end result will be individual likelihood ratios from each system tested comparing the pair of hypotheses referred to as Kinship Indices (KI). The individual KIs are multiplied across all tested loci/systems (STR, Y-STR haplotype, mtDNA haplotype) to provide a Combined KI for the overall evaluation. During reporting and testimony, it is crucial that the value obtained be correctly presented in the context of the specific association. Sample wording for various comparisons is provided in Appendix B.

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

As a working example, begin with the following pedigree:

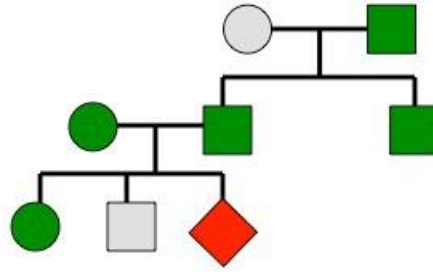


Figure 1. Example Pedigree

The red diamond represents the missing person from the pedigree and the green circles and squares (females and males, respectively) represent possible family reference samples obtained in these cases. Rarely will a pedigree contain all of the members shown in this example. In the case scenario below, different combinations of these family members will be used to demonstrate the statistical value various relationships can provide.

The case scenario is as follows:

- A young man went missing some number of years ago.
- The family filed a missing person report.
- Cold-case detectives began gathering family reference samples in more recent years.
- An association was made in a search of NDIS between an unidentified remains sample submitted from an adjoining state and the pedigree assembled from the family reference samples.

The family members indicated they were of African American origin in their sample submission documents and anthropological examination of the remains was consistent with the remains originating from a male of African descent. The unidentified remains sample yielded a full STR profile (20 loci including Amelogenin), a full Y-STR haplotype (17 loci), and a complete mtDNA haplotype (16000-16386; 52-399). The autosomal STR allelic composition for the remains and reference samples are provided in Table 2.

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

Table 2. Autosomal STR allelic composition of unidentified remains and potential family reference samples.

STR Locus	Unidentified Remains	Mother	Father	Sister	Paternal Grandfather	Paternal Uncle
CSF1PO	12,13	12,12	13,13	12,13	13,13	10,13
D10S1248	13,16	13,14	16,16	14,16	14,16	13,14
D12S391	18,23	18,23	18,18	18,18	18,20	18,18
D13S317	12,14	12,14	9,12	9,14	12,13	9,13
D16S539	12,12	12,13	11,12	12,12	11,11	11,12
D18S51	15,19	15,19	14,15	14,19	15,16	16,16
D19S433	12,12	12,15	12,14	14,15	14,14	12,14
D1S1656	13,15	13,15	15,15	15,15	14,15	14,15
D21S11	29,30	30,32.2	29,36	32.2,36	29,30	30,36
D22S1045	15,17	11,15	11,17	11,15	15,17	11,15
D2S1338	20,23	19,23	20,24	19,20	20,22	20,24
D2S441	11,14	10,11	10,14	10,11	11,14	11,11
D3S1358	16,16	16,18	16,17	16,16	15,16	15,18
D5S818	12,13	12,13	11,12	12,13	12,12	12,13
D7S820	8,12	10,12	8,8	8,12	8,11	8,10
D8S1179	11,12	11,13	11,12	11,13	11,12	11,14
FGA	20,23	23,23	20,20	20,23	20,22	20,23
TH01	7,9.3	7,9.3	7,11	7,7	9,3,11	8,9,3
TPOX	8,11	8,8	11,11	8,11	11,11	9,11
vWA	16,19	16,16	14,19	14,16	14,15	15,19
Amelogenin	XY	XX	XY	XX	XY	XY

Working pedigrees can be made from various combinations of the family references available. Additionally, lineage marker testing can provide additional strength to a putative association or exclude the unidentified remains from being a member of the pedigree. It is critical that the pedigree composition is verified to ensure that accurate relationships are depicted between the pedigree members. Potential contributors for lineage data and results from the assessment of Y-STRs and mtDNA are found in Table 3.

**SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023**

Table 3. Database statistics for lineage testing performed on the unidentified remains sample. Individuals that can contribute lineage data with these marker systems are indicated with an “X”.

	Unidentified Remains	Mother	Father	Sister	Brother	Paternal Grandfather	Paternal Uncle
Y-STR	X		X		X	X	X
mtDNA	X	X		X	X		

Using YHRD for Y-STR database search:
 Dataset: Y17, Kit: Yfiler
 0 observations in 7,120 haplotypes (US African American)
 Upper 95% CI: frequency 0.000421; LR = 2377

Using PopStats for mtDNA database search:
 0 observations in 2,449 haplotypes (African American)
 Upper 95% CI: frequency 0.0012; LR = 833

Utilizing the data presented, several testing scenarios are possible in this sample case. One possibility is that only a single reference sample from this set is available for comparison to the unidentified remains. Below are examples of statistical results that would be obtained from pairwise comparisons of the unidentified remains to the putative relatives (Table 4). Each of these calculations can be performed in the PopStats module of CODIS.

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

Table 4. Statistical results from kinship evaluations based on pairwise comparisons of a single family reference sample to the unidentified remains profile. NA indicates relative that cannot contribute the specified lineage marker results. Kinship indices shown below were generated using allele frequencies contained with the African American population of the FBI expanded CODIS database (Moretti et al. 2016).

Locus	Mother	Father	Sister	Paternal Grandfather	Paternal Uncle
	KI	KI	KI	KI	KI
CSF1PO	1.745980707	8.758064516	10.52171715	4.879032258	2.689516129
D10S1248	1.077380952	5.776595745	1.694148936	1.944148936	1.038690476
D12S391	8.060728745	2.198380567	1.349190283	1.049595142	1.599190283
D13S317	6.217200413	0.560950413	3.078125	0.780475207	0.5
D16S539	2.751269036	2.751269036	10.57075034	0.5	1.875634518
D18S51	4.371989712	1.489010989	1.691489362	1.244505495	0.5
D19S433	4.981651376	4.981651376	0.25	0.5	2.990825688
D1S1656	3.390441011	2.728643216	1.614321608	1.182160804	1.182160804
D21S11	1.475543478	1.371212121	0.25	1.9233778	1.237771739
D22S1045	1.165236052	1.020676692	0.832618026	1.592956372	1.082618026
D2S1338	2.340517241	3.085227273	1.792613636	2.042613636	2.042613636
D2S441	0.629930394	1.064705882	0.564965197	1.347318138	1.129930394
D3S1358	1.611275964	1.611275964	4.457486198	1.305637982	0.5
D5S818	1.706542449	0.654216867	2.480169515	1.154216867	1.353271224
D7S820	2.381578947	2.888297872	9.041573348	1.222074468	1.222074468
D8S1179	6.313953488	8.37077167	3.406976744	4.685385835	3.656976744
FGA	3.102857143	7.757142857	14.99965306	2.439285714	3.215
TH01	3.258628752	0.622706422	0.872706422	1.817961165	1.817961165
TPOX	1.422572178	2.306382979	2.822736919	1.653191489	1.076595745
vWA	1.853242321	3.719178082	0.71331058	0.5	2.359589041
Combined* STRs	5.3E+7	1.6E+7	1.0E+6	7.6E+2	1.0E+3
STRS & mtDNA	4.4E+10	NA	8.4E+8	NA	NA
STRS & Y-STR	NA	4.0E+10	NA	1.8E+6	2.4E+6
STRS & mtDNA & Y-STR	NA	NA	NA	NA	NA

*The combined kinship indices in the above table were truncated. The allele-specific KI values are provided for calculation purposes.

Having two family reference samples available for comparison in a pedigree provides

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

marked improvement in the weight of the statistical evaluation performed; this is demonstrated in Table 5. Although the Reverse Parentage calculation (using the mother and father) can be performed in the PopStats module of CODIS, other combinations require the use of pedigree-based software tools.²

The inclusion of additional family reference samples in a pedigree will generally improve the statistical strength of an evaluation. The selection of additional pedigree members can be strategically made to permit the inclusion of lineage markers, such as Y-STRs, as shown in Tables 4-6. The inclusion of the more distant paternal relatives alone does not substantially improve the power of the KI. There are additional scenarios possible and one often overlooked is the testing of multiple siblings³ of a missing person in lieu of having one or both parents available (Table 6). Similarly, the inclusion of a spouse and children of the missing person with a parent and/or sibling can greatly improve the statistical evaluation of the genetic data. Strategically selecting available family references in lieu of unavailable parent(s) to provide lineage markers (mtDNA and Y-STR) should be actively discussed with investigators when there is a need to request additional family reference samples.

² All pedigree calculations performed for this example utilized the KIn CALc 5.0 spreadsheet kindly provided by Steven Myers, California Department of Justice, Richmond, CA.

³ Additional sibling profiles not shown.

SWGAM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

Table 5. Statistical results from kinship evaluations based on pedigrees containing two family reference samples compared to the unidentified remains profile. NA indicates relative that cannot contribute specified lineage marker results.

Locus	Mother & Sister	Father & Sister	Mother & Father	Paternal Grandfather & Sister	Paternal Uncle & Sister	Paternal Uncle & Mother
	KI	KI	KI	KI	KI	KI
CSF1PO	16.16440203	19.67044394	30.58282336	10.97768496	10.37643026	8.518696193
D10S1248	6.762284701	2.888297872	12.44718845	1.64215915	3.130763068	0.538690476
D12S391	19.33447524	1.099190283	30.60822174	1.185108005	1.224190283	19.33447524
D13S317	3.108600207	6.626226756	6.34575155	3.140737991	1.607295133	3.108600207
D16S539	8.945115824	8.945115824	7.569481306	8.945115824	10.3540724	5.160375171
D18S51	2.185994856	9.330079495	8.585574	3.239390928	1.206117021	2.185994856
D19S433	2.490825688	2.490825688	24.81685043	0.179326288	1.095934627	14.8992509
D1S1656	7.223777469	1.364321608	11.05711393	1.455797349	1.193979077	4.459498987
D21S11	0.737771739	0.685606061	4.046566206	0.7116889	0.490550554	0.737771739
D22S1045	0.582618026	2.888996902	2.378658556	1.109647319	0.707618026	0.582618026
D2S1338	15.61231387	1.542613636	14.44205525	1.731417531	1.667613636	8.391286246
D2S441	0.314965197	1.873734134	1.341381193	0.780631934	0.562604367	0.314965197
D3S1358	3.401848216	3.401848216	2.596210233	4.704601453	3.929667207	0.805637982
D5S818	2.230169515	1.704006725	1.376898291	2.62698853	2.482351918	2.230169515
D7S820	8.06949888	15.20156775	13.75741881	9.399277661	8.794773337	4.630144177
D8S1179	3.156976744	4.185385835	25.97330867	4.330899999	3.281976744	3.156976744
FGA	25.62073469	27.94787755	48.13861224	15.48561625	15.34256337	13.58608163
TH01	4.912125902	0.311353211	3.282811526	2.611739556	1.742222989	3.270720139
TPOX	3.992282348	4.434187748	6.561992517	3.028619122	2.656444371	2.351784218
vWA	0.92662116	8.752127262	13.78507644	0.607970143	1.798947473	7.819159381
Combined* STRs	4.3E+11	8.3E+10	4.4E+18	2.0E+7	1.2E+7	5.0E+9
STRs & mtDNA	3.6E+14	6.9E+13	3.7E+21	1.6E+10	1.0E+10	4.1E+12
STRs & Y-STR	NA	1.9E+14	1.0E+22	4.8E+10	2.9E+10	1.1E+13
STRs & mtDNA & Y-STR	NA	1.6E+17	8.8E+24	4.0E+13	2.4E+13	9.9E+15

*The combined kinship indices in the above table were truncated. The allele-specific KI values are provided for calculation purposes.

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

Table 6. Statistical results from kinship evaluations based on pedigrees containing three or four family reference samples compared to the unidentified remains profile.

Locus	Mother, Sister & Paternal Grandfather	Mother, Sister & Paternal Uncle	Mother, Sister & Brother	Mother, 2 Sisters & Brother
	KI	KI	KI	KI
CSF1PO	16.94309726	16.1196703	16.94309726	18.27275609
D10S1248	7.141989605	6.492939463	7.215149915	7.934172755
D12S391	20.46682675	24.97134849	21.68454193	24.31023373
D13S317	3.172875775	1.554300103	3.027748721	2.878039066
D16S539	7.569481306	8.761759937	7.569481306	7.569481306
D18S51	4.292787	1.092997428	2.080437598	8.585574
D19S433	2.080797459	11.89993912	2.080797459	1.565414755
D1S1656	7.510492479	6.746848765	8.12335537	8.76597282
D21S11	2.023283103	0.36888587	0.734390567	4.046566206
D22S1045	0.837358968	0.291309013	2.378658556	2.378658556
D2S1338	16.45616121	15.53071105	16.60713338	18.20768743
D2S441	0.492335202	0.157482599	0.210364209	1.341381193
D3S1358	3.590440743	2.999029225	2.596210233	2.596210233
D5S818	2.430511196	2.491983048	1.376898291	1.376898291
D7S820	8.647789254	7.916374105	8.908846563	10.01373333
D8S1179	6.445582114	1.578488372	2.522793778	25.97330867
FGA	26.81784026	25.53215394	26.98427399	29.26946485
TH01	4.924217289	4.918171595	5.460578187	5.846480919
TPOX	4.450115471	3.884719779	4.450115471	5.004940631
vWA	0.812851842	3.90957969	13.78507644	13.78507644
Combined* STRs	1.2E+13	1.4E+11	9.4E+12	2.3E+16
STRS & mtDNA	1.0E+16	1.2E+14	7.8E+15	1.9E+19
STRS & Y-STR	3.0E+16	3.4E+14	2.2E+16	5.5E+19
STRS & mtDNA & Y-STR	2.5E+19	2.8E+17	1.8E+19	4.6E+22

*The combined kinship indices in the above table were truncated. The allele-specific KI values are provided for calculation purposes. These examples are not referring to the pedigree example at the beginning of the Appendix, but shown to demonstrate different KI values obtained when three and four family reference samples are used for the calculations.

Appendix B. Statistical Report Wording Examples

Reporting the results of a statistical evaluation from a missing person association encompasses the hypotheses tested and all of the genetic data utilized in the evaluation (the results may also be reported separately by technology). The following examples are independent of the example from Appendix A. Appropriate wording for statistical conclusions includes:

1. Mother and Father

The genetic data (autosomal STRs, mtDNA, & Y-STRs) are approximately 2.3 trillion times more likely to be observed if the unidentified remains originated from a biological child of John Smith and Jane Smith as opposed to if the unidentified remains originated from an unrelated individual from the African American population.

2. Mother, Sister, and Paternal Uncle

The genetic data (autosomal STRs, mtDNA, & Y-STRs) are approximately 1.63 billion times more likely to be observed if the unidentified remains originated from a biological child of Jane Smith, from a biological sibling of Judy Smith and a paternal nephew of Ralph Smith as opposed to if the unidentified remains originated from an unrelated individual from the African American population.

3. Mother and three full siblings (Two Sisters and Brother)

The genetic data (autosomal STRs, mtDNA, & Y-STRs) are approximately 483 billion times more likely to be observed if the unidentified remains originated from a biological child of Jane Smith and from a biological sibling of Judy Smith, Mary Smith and Jack Smith as opposed to if the unidentified remains originated from an unrelated individual from the African American population.

4. Example where technologies are reported separately

SWGDM Missing Persons Casework Guidelines
Draft for SWGDAM Comment 12052023

Based on the DNA typing results, and the comparison of the unidentified remains to Jeffrey Doe, the combined kinship index is approximately 170,000. Therefore, the DNA profile obtained from the unidentified remains is approximately 170,000 times more likely if the unidentified remains are from the brother of Jeffrey Doe than if the unidentified remains are from someone unrelated to this individual. These results provide strong evidence that the unidentified remains originated from Joe Doe.

The Y-STR typing results obtained from the unidentified remains and Jeffrey Doe are the same; therefore, the unidentified remains could have originated from Joe Doe. These results are 2,000 times more likely if the unidentified remains are from Joe Doe than if the unidentified remains are from an unknown, unrelated male.

Likelihood Ratio (LR)	Level of Support
2,000	Moderate Support for Inclusion

Along with the conclusions (e.g., the unidentified remains sample cannot be excluded from being a child of...etc.) and the statistical weight of the genetic evidence developed in the case, the following statements may be included in the report:

Investigators are strongly encouraged to evaluate all associated case information in addition to the provided genetic results before declaring identity of the remains.

In the event that an identification is rendered by the appropriate legal authority, a copy of the death certificate or written communication of identification is requested by the laboratory so that all genetic data obtained from the associated family reference sample(s) can be expunged from the CODIS database.