Guimarães *et al.*
Egyptian Journal of Forensic Sciences (2025) 15:11
https://doi.org/10.1186/s41935-025-00432-6

Egyptian Journal of Forensic Sciences

REVIEW

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Determination of sexual dimorphism through molecular methods: a scoping review

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Abstract

Background Sexual dimorphism is crucial in forensic investigations. Molecular methods involving amelogenin, a protein in tooth enamel, are used to determine sexual dimorphism by extracting DNA from teeth, amplifying the gene coding for amelogenin through PCR, and analyzing PCR product sizes to identify X and/or Y chromosomes.

Objective This scoping review explored scientific studies using the amelogenin gene to determine sex in forensic dentistry. It addressed the research question: do molecular methods determine sexual dimorphism for forensic identification?

Material and methods A literature review (1996–2024) was conducted using PubMed, MEDLINE (via BVS), and CINAHL (via EBSCO host). Inclusion and exclusion criteria guided the selection process, summarized in a PRISMA flowchart. A PCC (Population-Concept-Context) strategy was applied to formulate the research question.

Results Thirteen of the 1091 articles initially considered met the inclusion and exclusion criteria. These studies examined forensic dentistry and sex determination through amelogenin identification. They were categorized into "with treatment" (7 studies) and "without treatment" (6 studies), and all involved in vitro research.

Conclusion Molecular methods targeting the amelogenin gene on the X and Y chromosomes offer an accurate and reliable approach to determining sex.

Keywords Forensic dentistry, Analyses, Sex determination, Amelogenin

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Background

Forensic dentistry is a specialized discipline that serves as a crucial intersection between dentistry and the legal system, providing indispensable expertise in identifying individuals based on dental evidence. This field, forensic odontology, plays a pivotal role when traditional identification methods, such as fingerprints or facial recognition, are compromised or unavailable (Jeddy et al. 2017; Herrera-Escudero et al. 2024). The primary focus of forensic dentistry is the accurate identification of individuals, often using dental impressions or DNA extracted from teeth, especially in cases where the body is unrecognizable due to severe trauma or decomposition (Shanbhag 2016; Krishan et al. 2015; Herrera-Escudero et al. 2024).

Historically, identification has relied heavily on digital fingerprints, but these degrade rapidly after death, making them less reliable in specific forensic scenarios (Musse et al., 2009). In contrast, teeth are remarkably resilient, withstanding environmental factors that can obliterate other biological evidence (Herrera-Escudero et al. 2024). This resilience is due to the highly mineralized structure of teeth, particularly the enamel, which makes them one of the most durable components of the human body (Guimarães et al. 2017). Teeth can endure extreme conditions, including high temperatures ranging from 150 °C to 450 °C, and remain intact even when other tissues have decomposed or been destroyed (Yashoda et al. 2021).

In the aftermath of catastrophic events such as terrorist attacks, natural disasters, and severe accidents, bodies may suffer such extensive damage that they become visually unrecognizable. In these cases, forensic odontologists can extract DNA from the dental pulp or the hard tissues of the teeth, providing a reliable means of identification. The dental pulp, located inside the tooth and protected by enamel and dentin, contains a variety of cells, including fibroblasts, odontoblasts, and endothelial cells, as well as blood elements, all of which are excellent sources of DNA that are often well-preserved despite exposure to extreme conditions (Chowdhury et al. 2018).

Identifying individuals using dental evidence becomes even more critical when antemortem data, such as medical records or fingerprints, are unavailable. DNA profiling systems play a vital role in these scenarios, allowing forensic experts to compare DNA extracted from the teeth of unidentified individuals with samples from known relatives or personal items, such as toothbrushes or stored blood. This method has proven effective in many forensic investigations, providing a powerful tool for uncovering the identity of victims when other methods fail (Manjunath et al. 2011).

Furthermore, teeth are not only useful for identification but also for determining the sex of an individual, even when DNA samples are fragmented or degraded. Enamel, the outermost layer of the tooth, contains amelogenin, a protein that differs in size and nucleotide sequence between males and females. This difference allows forensic scientists to use PCR analysis to amplify specific regions of the *AMEL* gene, enabling accurate sex determination (Dutta et al. 2017). Amelogenin-based tests are particularly valuable in cases where fire, explosions, or prolonged environmental exposure have damaged biological samples (Miksik et al. 2023). While DNA profiling and fingerprint analysis serve as primary and definitive identification techniques, sex determination can function as a valuable secondary identifier. It can corroborate findings from primary methods, aid in narrowing down potential matches within a database, and provide crucial information in cases where primary methods are inconclusive or unavailable.

The study of teeth provides insights into DNA degradation and preservation processes and their forensic applications. For example, permanent teeth are more robust in terms of DNA preservation than primary teeth due to their higher levels of mineralization and lower organic content (Williams et al. 2004). This understanding is crucial in forensic investigations, mainly when dealing with ancient or highly degraded samples.

Overall, forensic dentistry is an essential tool in forensic science. It offers reliable methods for identifying individuals and determining sex, even under the most challenging circumstances. Its importance continues growing as DNA technology and dental science advances provide new opportunities for solving complex forensic cases.

This work intends to conduct a scoping review to determine the sex of a person whose identity cannot be determined by other methods. To do this, molecular techniques based on the *AMEL* gene, present on the X and Y chromosomes, will be evaluated.

Methods

This scoping review followed the guidelines of the Joanna Briggs Institute (JBI) methodology (Aromataris & Munn 2020). The items identified in the reports prepared for the guidance of systematic reviews and extension of meta-analyses (PRISMA-ScR) were used (Page et al. 2021). The protocol was registered in the OSF (https://osf.io/3jnmc/) (https://doi.org/10.17605/OSF.IO/3JNMC) (accessed on 17 October 2024)). A previous protocol for this scoping review was published elsewhere (Lopes Cardoso et al. 2024).

The search produced 2704 results. Table 1 shows that 2271 papers were obtained from the PubMed search, 301 were in MEDLINE (via BVS), and 132 were selected in CINAHL (via EBSCO host).

The descriptors used were "amelogenin", "analyses, sex determination", "analysis, sex determination" and "human identification" with the Boolean operators AND/OR to form the combination: ("amelogenin"[Mesh] OR "amelogenin"[tiab]) AND (("Analyses, Sex Determination"[Mesh] OR "Analysis, Sex Determination"[Mesh] OR "Sex Determination Analyses"[Mesh] OR "Sex Determination"[tiab]) AND ("Human Identification"[Mesh] OR "Human Identifications"[Mesh] OR "Identification, Human"[Mesh] OR "Identifications, Human"[Mesh] OR "Human Identification"[tiab] OR "Human Identifications"[tiab] OR "Identification, Human"[tiab] OR "Identifications, Human"[tiab]).

However, after removing duplicates (47 duplicates), 2657 articles were identified. Of these, 853 articles were

Table 1 Search strategy

Database (electronic address)	Articulation of keywords	Number of articles
PubMed (https://pubmed.ncbi.nlm.nih.gov/advanced/)	("amelogenin"[Mesh] OR "amelogenin"[tiab]) AND (("Analyses, Sex Determination"[Mesh] OR "Analysis, Sex Determination"[Mesh] OR "Sex Determination Analyses"[Mesh] OR "Sex Determination"[tiab]) AND ("Human Identification"[Mesh] OR "Human Identifications"[Mesh] OR "Identification, Human"[Mesh] OR "Identifications, Human"[Mesh] OR "Human Identification"[tiab] OR "Human Identifications"[tiab] OR "Identification, Human"[tiab] OR "Identifications, Human"[tiab]))	**2271**
MEDLINE (via BVS) (https://bvsms.saude.gov.br/minibanners/medline/)	("amelogenin"[Mesh] OR "amelogenin"[tiab]) AND (("Analyses, Sex Determination"[Mesh] OR "Analysis, Sex Determination"[Mesh] OR "Sex Determination Analyses"[Mesh] OR "Sex Determination"[tiab]) AND ("Human Identification"[Mesh] OR "Human Identifications"[Mesh] OR "Identification, Human"[Mesh] OR "Identifications, Human"[Mesh] OR "Human Identification"[tiab] OR "Human Identifications"[tiab] OR "Identification, Human"[tiab] OR "Identifications, Human"[tiab]))	**301**
CINAHL (via EBSCO host) (https://web.p.ebscohost.com/ehost/search/basic?vid=1&sid=be6a4f61-14fd-424f-8feb-e966412165d1%40redis)	("amelogenin"[Mesh] OR "amelogenin"[tiab]) AND (("Analyses, Sex Determination"[Mesh] OR "Analysis, Sex Determination"[Mesh] OR "Sex Determination Analyses"[Mesh] OR "Sex Determination"[tiab]) AND ("Human Identification"[Mesh] OR "Human Identifications"[Mesh] OR "Identification, Human"[Mesh] OR "Identifications, Human"[Mesh] OR "Human Identification"[tiab] OR "Human Identifications"[tiab] OR "Identification, Human"[tiab] OR "Identifications, Human"[tiab]))	**132**

excluded after reading the title or abstract or because the full text was unavailable. Then, 1804 articles were selected for full reading, and 1791 were excluded after complete reading for failing to meet the inclusion and exclusion criteria.

Thus, 12 articles were selected to carry out the scoping review. Moreover, the authors found 12 more publications by citation search. After applying inclusion and exclusion criteria, 1 article was selected from these.

As reflected in the PRISMA 2000 flow diagram, 13 articles were selected for this scoping review. The bibliographic search strategy used is presented in Table 1.

This scoping review considered the PCC criteria. These criteria define the population (P) "Molecular methods", Concept (C) "Determination of sexual dimorphism", and Context (C) "Forensic identification" that formulate the question this work aims to answer.

In alignment with the aim of the scoping review, the following research question was developed: can molecular methods effectively determine sexual dimorphism for forensic identification?

Sources of information and search strategy

The final search was conducted on April 16, 2024, after several preliminary searches. The investigation was carried out using three bibliographic databases in the health domain. Table 1 presents the terms selected for the study and the Boolean operators AND/OR. An additional article, identified through other sources, was also included.

The search strategy was developed to identify studies that directly correlate "Forensic Dental Medicine: with "sexual dimorphism." The electronic search results were exported to Rayyan® (Mourad Ouzzani et al. 2016), and duplicates were eliminated. The developer of the software is Rayyan Systems Inc. from Cambridge, MA, USA. Software AI-driven was not used to select the articles. Rayyan software was used as a support tool, only to gather all the articles found in the different databases described above and identify duplicates. After two independent reviewers manually removed duplicates, screen study titles, and abstracts on the predefined inclusion and exclusion criteria. Full-text articles of potentially eligible studies were retrieved and independently assessed by the same two reviewers. A third reviewer was involved in resolving disagreements.

Although forensic dentistry and sexual dimorphism are distinct concepts, they are related because the first may be useful in the second. This review focuses on them.

Therefore, articles were included if they met all the following eligibility criteria:

- Population: molecular methods.
- Language: articles published in Portuguese, English, French, or Spanish.
- Timeline: no restrictions.

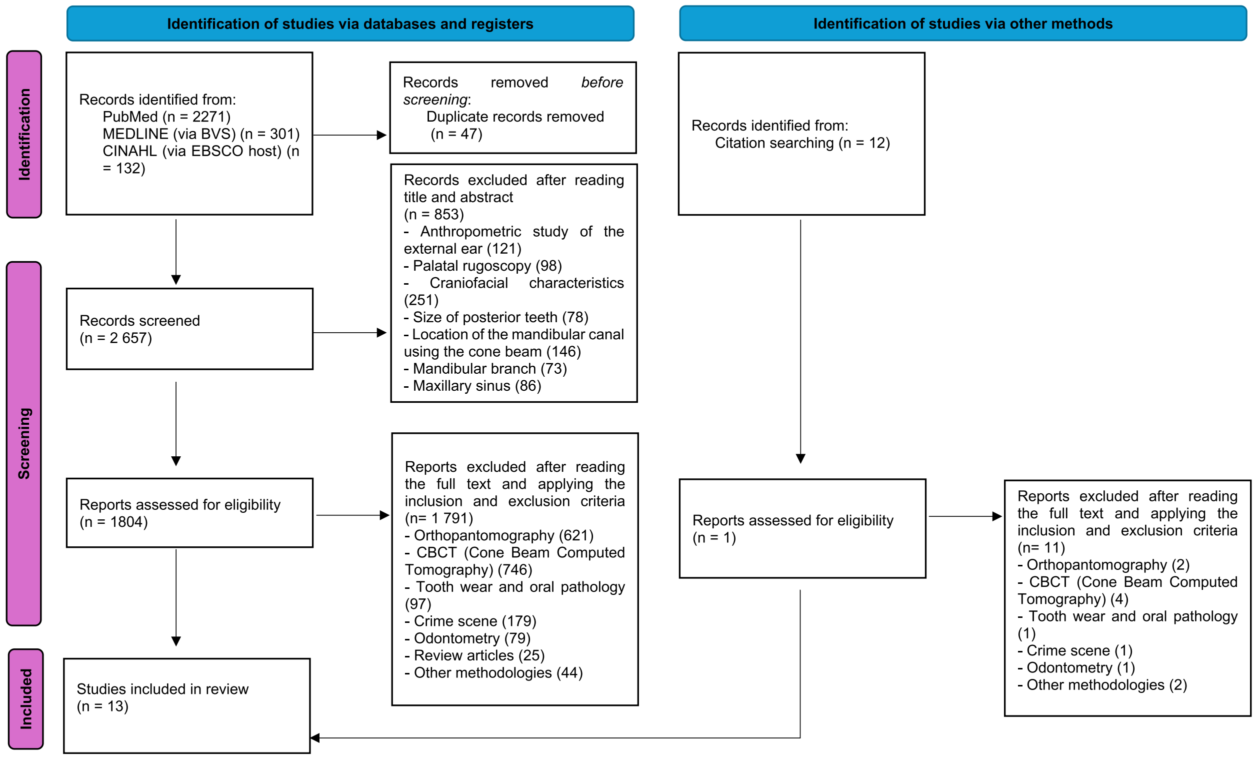

Fig. 1 The flowchart of the article's selection process was adapted from the PRISMA 2000 flow diagram (Page et al. 2021)

Inclusion criteria

All types of scientific work that addresses the topic of the relationship between forensic dentistry and sex determination through the identification of amelogenin. Articles that used teeth as a sample and in vitro studies.

Exclusion criteria

After thoroughly reading the articles retained for eligibility purposes, 1791 articles were excluded for not meeting the inclusion criteria; that is, they did not make reference to amelogenin to determine the sex of unidentifiable cadavers and made reference to other criteria that were excluded from this study, such as orthopantomography, CBCT (cone beam computed tomography), tooth wear and oral pathology, crime scene, odontometry, review articles, other methodologies (blood sample, bone, and muscles samples, buccal swabs and PCR on different genes).

Results

Molecular methods based on the amelogenin gene present on the X and Y chromosomes determine the sex of an individual.

A total of 2704 articles were identified, and 47 duplicates were removed. Figure 1 shows the PRISMA 2000 flow diagram that tracks progress.

The selected studies were independently reviewed with full-text reading, and the following information was extracted: the article's name, author, year and place of publication, methodology, main conclusions, and relevant final considerations. The data mapping is presented in Table 2.

PRISMA guided the collection, interpretation, and communication of results. Thus, several articles were analyzed using the search in PubMed, MEDLINE (via BVS), CINAHL (via EBSCO host), and other sources to obtain only the relevant data for this scoping review. A total of 13 articles relevant to this study were found.

The essential data on the methodologies and results of these 13 articles are summarized in Table 3.

In this scooping review, 13 articles have been explored from various aspects to enable sex determination from dental tissue in forensic contexts. Papers Zapico et al. (2013), Praveen Kumar et al. (2016), Komuro et al. (1998), Srivastava et al. (2017), Sivagami et al. (2000), and Nogami et al. (2008) focused on standard DNA extraction methods and sex determination without controlled variations in environmental or storage conditions.

In contrast, references Nayar et al. (2017), Williams et al. (2004), Dutta et al. (2017), Chowdhury et al. (2018), Alvarez García et al. (1996), Urbani et al. (1999) and Musse et al. (2009) were selected specifically to use

Table 2 General characteristics of the selected studies

Author/year	Article title	Type of study	Place of publication
Nayar et al. (2017)	Determination of age, sex, and blood group from a single tooth	In vitro	Journal of Forensic Dental Sciences
Williams et al. (2004)	Sex determination by PCR analysis of DNA extracted from incinerated, deciduous teeth	In vitro	Science & Justice
Dutta et al. (2017)	Amelogenin Gene—The Pioneer in Gender Determination from Forensic Dental Samples	In vitro	Journal of Clinical and Diagnostic Research
Chowdhury et al. (2018)	Sex Determination by Amplification of Amelogenin Gene from Dental Pulp Tissue by Polymerase Chain Reaction	In vitro	Indian Journal of Dental Research
Alvarez García et al. (1996)	Effect of environmental factors on PCR-DNA analysis from dental pulp	In vitro	Internacional Journal of Legal Medicine
Urbani et al. (1999)	The effect of temperature on sex determination using DNA-PCR analysis of dental pulp	In vitro	The Journal of Forensic Odonto-Stomatology
Zapico et al. (2013)	Sex determination from dentin and pulp in a medicolegal context	In vitro	Journal of the American Dental Association
Praveen Kumar et al. (2016)	DNA isolation from teeth by organic extraction and identification of sex of the individual by analyzing the AMEL gene marker using PCR	In vitro	Journal of Forensic Dental Sciences
Komuro et al. (1998)	Gender determination from dental pulp by using capillary gel electrophoresis of amelogenin locus	In vitro	The Journal of Forensic Odonto-Stomatology
Srivastava et al. (2017)	Sex determination from mesiodens of Indian children by amelogenin gene	In vitro	Journal of Forensic Dental Sciences
Musse et al. (2009)	Freshwater and salt-water influence in human identification by analysis of DNA: an epidemiologic and laboratory study	In vitro	Brazilian Journal of Oral Sciences
Sivagami et al. (2000)	A simple and cost-effective method for preparing DNA from the hard tooth tissue, and its use in polymerase chain reaction amplification of amelogenin gene segment for sex determination in an Indian population	In vitro	Forensic Science International
Nogami et al. (2008)	Rapid and simple sex determination method from dental pulp by loop-mediated isothermal amplification	In vitro	Forensic Science International: Genetics

varied experimental treatments to simulate different environmental and preservation conditions. These studies involved submitting tooth samples to normal environmental conditions, salt water, immersion in salt water under different periods depending on the study, incineration varying time of exposure and temperature depending on the study, desiccation at room temperature, burying in soil and/or sand, river water and cadaver teeth embedded in bone and surrounded by soft tissue.

This approach enabled us to compare results obtained under normal and extreme conditions, enriching our understanding of the challenges and possibilities of determining sex from dental tissue.

Nayar et al. (2017) results indicated high specificity for PCR sex determination performed directly on pulp tissue, with no significant impact on time (2 days and 6 weeks) or environmental (normal and saltwater) conditions.

Williams et al. (2004) results showed that sex determination was possible for teeth incinerated for 15 min at temperatures up to 200 °C, but not beyond.

In 2017, Dutta et al. showed that it was possible to determine sex in most samples. However, samples exposed to seawater or incinerated at temperatures above 800 °C required more PCR cycles for adequate amplification.

The 2018 study by Chowdhury et al. showed complete sensitivity and specificity in sex determination using teeth subjected to immersion in salt water, desiccation at room temperature, burial in soil (all for 30 to 90 days), and incineration at 150 °C and 205 °C. However, no DNA could be recovered at extreme temperatures (350 °C).

Alvarez García et al. (1996) showed that sex determination was possible for all samples stored at different temperatures, buried or left outdoors. Similarly, sex determination was successful for all samples immersed in water for 3 and 6 months, as well as for all samples incinerated, except those exposed to 500 °C for 2 min. Sex determination was also 100% successful for old and forensic samples (10 to 30 years old).

Urbani et al. (1999) were able to determine sex with 100% accuracy for samples subjected to 100 °C for 15 min, but above 100 °C or for longer durations, the accuracy of sex determination decreased. Nevertheless, it

Table 3 Essential data taken from the selected publications

Author (year)	Type of study	Title	Methodology	Results
Nayar et al. (2017)	In vitro	Determination of age, sex, and blood group from a single tooth	–60 teeth -With treatment: normal environmental conditions and under saline water (for 2 days and 6 weeks each) -PCR performed directly in pulp tissue -PCR followed by gel electrophoresis (PAGE)	-High specificity in sex determination using PCR in all samples -No significant effect of time or environmental conditions on sex determination
Williams et al. (2004)	In vitro	Sex determination by PCR analysis of DNA extracted from incinerated, deciduous teeth	–84 deciduous primary molars and 2 mandibular decidual teeth of a man who died in a fire -With treatment: incineration for 15 min at temperatures ranging from 100 °C to 500 °C -DNA extracted from pulp tissue with kit -PCR followed by gel electrophoresis (agarose)	-Successful sex determination in control teeth and teeth incinerated to temperatures of 100–200 ºC -Successful sex identification in teeth from a fire victim -Sex determination not possible in teeth incinerated at temperatures above 200ºC
Dutta et al. (2017)	In vitro	Amelogenin Gene—The Pioneer in Gender Determination from Forensic Dental Samples	–50 teeth (premolars and molars) with and without carious lesions -With treatment: salt water; desiccation at RT; buried in soil; incineration at 500–1050 ºC -DNA extracted from pulp tissue with phenol/chloroform method -PCR followed by gel electrophoresis (agarose)	-Possible sex determination in most samples -Samples submitted to seawater or incinerated above 800 ºC required an increase in PCR cycles for adequate amplification
Chowdhury et al. (2018)	In vitro	Sex Determination by Amplification of Amelogenin Gene from Dental Pulp Tissue by Polymerase Chain Reaction	–130 premolars -With treatment: salt water (30 to 90 days); desiccation at RT (30 to 90 days); buried in soil (30 to 90 days); incineration at 150 ºC, 250 ºC and 350 ºC -DNA extracted from pulp tissue with phenol/chloroform method -PCR followed by gel electrophoresis (agarose)	-Sex determination demonstrates complete sensitivity and specificity -at extreme temperatures (350 °C) no DNA could be recovered
Alvarez García et al. (1996)	In vitro	Effect of environmental factors on PCR-DNA analysis from dental pulp	–570 teeth; 6 teeth extracted 10–30 years before; 4 teeth from forensic casework. All teeth were premolars and molars -With treatment: salt and river water (15 days to 6 months); kept at 4 ºC, 20 ºC and 40 ºC (2 weeks to 36 months); buried in soil or in sand (2 weeks to 6 months); outdoors (2 weeks to 6 months); incineration for 1–2 min at 75 ºC, 100 ºC, 200 ºC, 300 ºC, 400 ºC, and 500 ºC -DNA extracted from pulp tissue with kit -PCR followed by gel electrophoresis (PAGE)	-Sex determination in all samples kept at different temperatures, buried or left outdoors -Sex determination in all samples kept in water for 3 and 6 months -Poorest DNA isolation in water-immersed samples -Sex determination in all incinerated samples. Exception: no sex determination when exposed to 500 ºC for 2 min –100% sex determination in old samples (10 to 30 years old) –100% sex determination in forensic casework samples

Table 3 (continued)

Author (year)	Type of study	Title	Methodology	Results
Urbani et al. (1999)	In vitro	The effect of temperature on sex determination using DNA-PCR analysis of dental pulp	–88 impacted 3rd molars, with and without carious lesions; 6 teeth in bone sockets from a cadaver -With treatment: incineration for 15 or 30 min at 100 ºC, 200 ºC, and 300 ºC; cadaver teeth embedded in bone and surrounded by soft tissue incinerated for 15 min at 150 ºC, 250 ºC, and 350 ºC -DNA extracted from pulp tissue with phenol/chloroform method -PCR followed by gel electrophoresis (agarose)	-Sex determination with 100% accuracy in samples submitted to 100 °C for 15 min -Above 100 ºC or longer time, the accuracy of sex determination decreased -Remained possible at 250 °C for teeth embedded in bones
Zapico et al. (2013)	In vitro	Sex determination from dentin and pulp in a medicolegal context	–2 incisors and 12 molars with periodontal disease -Without treatments -DNA extracted from pulp and dentin tissues with kit -PCR followed by gel electrophoresis (agarose)	-Possible to determine sex using pulp or dentin samples -Similar amounts of DNA are obtained using different extraction methods -Efficiency of DNA isolation from dentin depends on the type of tooth used
Praveen Kumar et al. (2016)	In vitro	DNA isolation from teeth by organic extraction and identification of sex of the individual by analyzing the AMEL gene marker using PCR	–40 teeth -Without treatments -DNA extracted from hard tooth tissues with phenol/chloroform method -PCR followed by gel electrophoresis (agarose)	-Enough good-quality DNA isolated from teeth -Sex determination in all samples -PCR was sensitive and effective for sex determination
Komuro et al. (1998)	In vitro	Gender determination from dental pulp by using capillary gel electrophoresis of amelogenin locus	–20 teeth -Without treatments -DNA extracted from pulp tissue with phenol/chloroform method -PCR followed by PAGE or CGE	-Sex determination in all samples
Srivastava et al. (2017)	In vitro	Sex determination from mesiodens of Indian children by amelogenin gene	–8 mesiodens -Without treatments -DNA extracted from hard tooth tissues with phenol/chloroform method -PCR followed by gel electrophoresis (agarose)	-Successful DNA isolation and sex determination from 75% of samples
Musse et al. (2009)	In vitro	Freshwater and salt-water influence in human identification by analysis of DNA: an epidemiologic and laboratory study	–40 teeth and saliva samples as control -With treatments: immersion in fresh and saltwater for 1 month -DNA extracted from hard tooth tissues with phenol/chloroform method -PCR followed by gel electrophoresis (agarose)	-Successful DNA isolation from 37.5% of teeth samples; successful DNA isolation from all saliva samples -Sex determination in 83.3% of teeth samples and in 100% of saliva samples -Water interfered directly with DNA preservation
Sivagami et al. (2000)	In vitro	A simple and cost-effective method for preparing DNA from the hard tooth tissue, and its use in polymerase chain reaction amplification of amelogenin gene segment for sex determination in an Indian population	–10 teeth -Without treatments -DNA isolated from hard tooth tissues by ultrasonication and phenol/chloroform extraction -PCR followed by gel electrophoresis (agarose)	-Sex determination in all samples

Table 3 (continued)

Author (year)	Type of study	Title	Methodology	Results
Nogami et al. (2008)	In vitro	Rapid and simple sex determination method from dental pulp by loop-mediated isothermal amplification	−32 teeth -Without treatments -LAMP method on pulp tissue (without DNA isolation) -PCR followed by PAGE	-Sex determination in all samples

remained possible to determine the sex for teeth embedded in bone up to a temperature of 250 °C.

Finally, the study by Musse et al. (2009) stated successful DNA isolation from 37.5% of tooth samples, while all saliva samples achieved successful DNA isolation. Sex determination was successful in 83.3% of tooth samples and 100% of saliva samples. Water directly impacted DNA preservation, affecting the ability to extract DNA from tooth samples.

Concerning the publications without treatment, the 2013 study by Zapico et al. examined sex determination from dentin and pulp in a forensic context. Results showed that it was possible to determine gender using pulp or dentin samples. Similar amounts of DNA were obtained using different extraction methods. However, the efficiency of DNA isolation from dentin depended on the type of teeth used.

Moreover, Praveen Kumar et al. (2016) observed sufficient good-quality DNA could be isolated from the teeth using the phenol/chloroform method without further sample processing. Sex determination was successful for all samples, and the PCR method proved sensitive and effective.

Komuro et al. (1998) stated that sex determination was successful for all dental pulp samples using capillary gel electrophoresis of the amelogenin locus. DNA extraction was carried out using the phenol/chloroform method without further sample processing.

In 2017, Srivastava et al. showed successful DNA isolation and sex determination from 75% of the mesiodens samples submitted to phenol/chloroform DNA extraction.

Sivagami et al. (2000) simple and cost-effective method for preparing DNA from hard tooth tissue successfully determined the sex of all the studied samples.

Finally, Nogami et al. (2008) used a rapid and straightforward method for determining sex from dental pulp using LAMP and were able to determine sex for all analyzed samples.

Discussion

The scoping review of scientific literature highlights the applicability and effectiveness of biological methods for sex determination based on identifying and analyzing sex-specific genetic markers, including the *AMEL* gene. These methods, particularly PCR (polymerase chain reaction), have proven advantageous over traditional approaches due to their speed, precision, and ability to work with even low-quality or degraded samples. By amplifying specific DNA sequences, PCR allows for detecting the *AMELX* and *AMELY* genes, which are pivotal in determining the sex of individuals from whom samples are taken. This is especially beneficial in forensic cases where DNA quantities might be limited.

The review categorized the 13 selected studies into two groups: those involving samples "with treatment" and those "without treatment," where "treatment" refers to conditions mimicking forensic scenarios. Examples of treatments included immersion in various water types, incineration, exposure to environmental elements, and burial. The studies utilized different methods for DNA extraction from dental tissues, including commercial kits, phenol/chloroform extraction, and direct PCR or LAMP (loop-mediated isothermal amplification) techniques.

Comparison of DNA extraction methods and samples

The most common DNA extraction method across the studies was phenol/chloroform extraction. However, other approaches, like using commercial kits or direct PCR on dental pulp tissue, were also employed. Differences in the types of dental tissues used for DNA extraction (e.g., pulp versus hard tissues like enamel and dentin) were noted, with varying success rates and DNA yields. For instance, Zapico et al. (2013) found that DNA yields varied depending on the tooth type and tissue used, with smaller teeth like incisors yielding less DNA. Despite these differences, sex identification was successful in all samples.

Similarly, Srivastava et al. (2017) and Praveen Kumar et al. (2016) used PCR after phenol/chloroform DNA extraction, focusing on different aspects of dental tissues. While Srivastava et al. reported a 75% success rate in sex identification, Praveen Kumar et al. emphasized the resistance of dental tissues to environmental degradation, making them reliable sources of DNA for forensic analysis.

Several studies also explored the effects of environmental conditions on DNA extraction and amplification. For example, Dutta et al. (2017) demonstrated a 100% DNA recovery rate from tooth samples, even under extreme conditions. However, they noted that samples subjected to extreme conditions required additional PCR cycles to detect small amounts of DNA. The study by Praveen Kumar et al. (2016) reported DNA yields of 55 to 86 μg per tooth, highlighting the purity of DNA extracted from dental tissues for forensic evidence.

Other studies focused on enhancing DNA extraction methods. Sivagami et al. (2000) used ultrasonication to disintegrate hard tooth tissues before DNA extraction, showing comparable PCR results for the AMEL gene between tooth-derived DNA and control samples. However, they should have reported specific DNA yields, making it difficult to assess the impact of ultrasonication on extraction efficiency.

Despite the overall effectiveness of PCR in sex determination, certain limitations were noted. Praveen Kumar et al. (2016) and Srivastava et al. (2017) pointed out that PCR requires expensive reagents and equipment and is prone to contamination, leading to false positives. They also emphasized the importance of magnesium ion concentration in PCR reactions, as inadequate levels can affect the results.

Effects of different treatments on DNA isolation and sex determination

Studies involving treated samples explored the impact of various environmental factors on DNA extraction and sex determination. Nayar et al. (2017) demonstrated that the PCR technique remained highly specific and accurate for sex determination, even after dental pulp samples were exposed to salt water for up to 6 weeks. This study underscores the robustness of dental pulp as a DNA source for forensic analysis.

Alvarez García et al. (1996) evaluated DNA analysis under various environmental conditions, including temperature variations, immersion in water, and burial. They found that sex could be determined in all outdoor and buried samples, with no significant differences between those buried in sand or soil. They also reported that even after incineration at temperatures up to 500 °C, sex determination was possible using the AMEL gene, although STR analysis was less reliable at higher temperatures.

Similarly, Dutta et al. (2017) investigated the effects of extreme conditions, such as immersion in salt water and incineration at temperatures up to 1050 °C. While sex determination was generally successful, higher PCR cycles were needed for severely treated samples. They suggested the LAMP method as a faster alternative to PCR, capable of providing results within 30 min under isothermal conditions.

Chowdhury et al. (2018) also tested teeth under various conditions, including immersion in salt water, desiccation, burial, and incineration. They concluded that sex identification was reliable for most samples, except those exposed to extremely high temperatures (350 °C), which did not allow for successful DNA isolation. This finding contrasts with Alvarez García et al. (1996), who reported positive results even at 500 °C, indicating some variability in outcomes depending on the specific methodologies and conditions used.

Studies by Williams et al. (2004) and Urbani et al. (1999) focused on the effect of incineration temperature on sex determination from teeth. Both studies found that while sex identification was possible, the results varied with temperature and sample protection. Williams et al. (2004) achieved positive sex identification up to 200 °C, but higher temperatures significantly reduced success rates. In contrast, Urbani et al. (1999) reported accurate results at lower temperatures, with diminishing accuracy at higher temperatures, especially without soft tissue and bone protection.

Although the *AMEL* gene is a widely used and effective marker in forensic science, several limitations must be acknowledged. Variations in the primer binding sites of the *AMEL* gene can lead to allele dropouts, resulting in the loss of the X allele in males or deletions of the Y chromosome. This can compromise the accuracy of sex determination and lead to misidentification. Moreover, relying solely on the *AMEL* gene can restrict the robustness of forensic identification. The absence of additional markers may increase the risk of errors, especially in degraded or low-quality samples.

Another limitation of the studied sex determination methods is that teeth exposed to extreme temperatures (above 250 °C) show reduced DNA yield. At 350 °C, no DNA can be extracted, limiting the ability to perform forensic identification in cases involving fire or extreme heat exposure. Moreover, while teeth are generally resilient, prolonged exposure to harsh environments, salt water, or acidic conditions may degrade DNA over time, reducing the effectiveness of PCR amplification.

PCR analysis often produces false negatives or positives due to contamination, low DNA concentrations, or technical errors during amplification, which can limit the accuracy of the results.

Finally, this scoping review is limited by including studies from specific databases and publications, potentially omitting relevant research in grey literature or unpublished works. Language restrictions and focusing on studies published within a specific timeframe may have excluded significant findings from earlier or non-English studies.

To mitigate these limitations, recommendations include using complementary genetic markers such as *Y-STR* and the *SRY* gene and employing advanced techniques like ultrasonication to enhance DNA extraction and quality.

Conclusions

The research reviewed underscores the significance of teeth as a resilient and valuable source of DNA for forensic identification, particularly in sex determination. Despite the limitations associated with environmental exposure, genetic variability, and methodological constraints, advances in PCR techniques and complementary genetic markers have enhanced the reliability of forensic analyses. The *AMEL* gene remains a cornerstone of sex determination, but integrating additional markers and innovative extraction techniques bolsters its effectiveness. Further refinement of methodologies and expanding genetic tools will strengthen forensic practices, ensuring greater accuracy and consistency in identification processes.

Abbreviations

AMEL	Amelogenin
APLP	Amplified product-length polymorphism
bp	Base pair, the unit of length in genetics representing the length of a DNA double helix segment
CGE	Capillary gel electrophoresis
CGTA	Canonical sequence of nucleotide bases (cytosine, guanine, thymine, adenine)
D1S80	DNA segment on chromosome 1
DNA	Deoxyribonucleic acid
DYS14	Y-chromosome-specific gene
DYZ3	Y-chromosome-specific alpha satellite DNA
HLADQA1	Human leukocyte antigen DQ Alpha 1 gene1
HUMFES/FPS	Human feline sarcoma oncogene homolog
HUMTH01	Human tyrosine hydroxylase gene
LAMP	Loop-mediated isothermal amplification
Mg^{2+}	Magnesium ion
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
SNP	Single nucleotide polymorphism
SRY	Gene coding for the sex-determining region Y protein
STR	Short tandem repeats
UV	Ultraviolet
ZF	Zinc finger proteins

Authors' contributions

Maria Inês Guimarães—Conceptualization; Validation; Literature search; Writing-initial draft preparation; Writing-editing; Writing-final text revision. The author has read and agree to the published version of the manuscript. Maria Teresa Moreira—Conceptualization; Validation; Literature search; Writing-initial draft preparation; Writing-editing; Writing-final text revision. The author has read and agree to the published version of the manuscript. Clarisse Dupuis—Conceptualization; Literature search; Writing-final text revision. The author has read and agree to the published version of the manuscript. Inês Lopes Cardoso—Conceptualization; Validation; Literature search; Writing-initial draft preparation; Writing-editing; Writing-final text revision. The author has read and agree to the published version of the manuscript.

Funding

Not applicable.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All author consents to publication.

Competing interests

The authors declare no competing interests.

Received: 11 October 2024 Accepted: 4 March 2025
Published online: 17 March 2025

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