forensic sciences

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Review

Amelogenin-Based Molecular Methods for Sexual Dimorphism Identification: Protocol of a Scoping Review

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Abstract: Forensic dentistry and sexual dimorphism are distinct concepts. Still, they are related due to the usefulness that the first may have in the second, and this review focuses on them. A scoping review will be performed according to the Joanna Briggs Institute's methodology. Scientific databases and grey literature will be used, and the following keywords will be applied: amelogenin, analyses, sex determination, and human identification. This scoping review will include in vitro studies concerning the goal of this review. This scoping review will deepen our knowledge concerning using teeth and amelogenin genes in sex identification in a forensic context. According to the available data, it will help implement guidelines for human remains identification. This protocol was registered with the Open Science Framework.

Keywords: amelogenin; analyses, sex determination; analysis, sex determination; human identification

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1. Introduction

Amelogenins are the main protein component present in the enamel matrix and are involved in amelogenesis, the development of enamel. Amelogenin is a type of extracellular matrix protein that, together with ameloblastin, enamelin, and tuftelin, directs the mineralization of enamel to form a highly organized matrix of rods, interstem crystals, and proteins. The mineralization process involves the formation of hydroxyapatite crystals, which are critical for the hardness and durability of enamel. These proteins play a crucial role in regulating crystal nucleation and growth, ultimately determining the structural integrity and functionality of the enamel [1,2].

The amelogenin system is paramount in forensic science, particularly in the context of genetic sex determination. This significance is underscored by its legal standing in several European countries, where it is the sole genetic locus authorized for use in such analyses according to current regulatory frameworks. The exclusive reliance on the amelogenin system in these jurisdictions stems from its reliability and established efficacy in differentiating between male and female DNA samples. This regulatory preference ensures consistency and standardization in forensic practices, enhancing the accuracy and legal defensibility of genetic sex determination in forensic casework [3,4]. Moreover, the standardization of forensic sex determination methods and results interpretation strategies worldwide is essential for the unequivocal identification of human remains in different forensic contexts. This standardized approach will enable a more reliable analysis of forensic medicine challenges.

It is important to note that developing human enamel contains approximately 70% proteins, 90% of which are amelogenins. These proteins can be preserved in the hard tissues of teeth (enamel) for thousands of years, making them invaluable for long-term studies. The high preservation potential of amelogenin in enamel is due to the protective nature of enamel itself, which is the most complex tissue in the human body. This resilience to environmental factors, such as temperature, humidity, and microbial activity, ensures that amelogenins can be reliably extracted even from ancient specimens, thus providing a rich source of genetic and proteomic information for researchers [1,2].

The amelogenin protein is encoded by the AMELX gene, present on the X chromosome, and by the AMELY gene, present on the Y chromosome. The presence of these genes on sex chromosomes makes them particularly useful in distinguishing between male and female individuals. Males possess both AMELX and AMELY genes, whereas females have two copies of the AMELX gene. Since the X and Y copies of this gene do not undergo homologous recombination, this gene is the most preferred genetic marker for sex identification [1]. This genetic distinction allows for a straightforward method of sex determination by analyzing the presence of these genes in extracted DNA samples.

The historical context of amelogenin research provides a backdrop for understanding its significance in modern forensic science. Early studies on enamel proteins focused primarily on their role in tooth development and the structural properties of enamel. However, with the advent of molecular biology techniques and the growing interest in forensic applications, researchers began to explore the potential of amelogenins as genetic markers for sex determination. The discovery of the AMELX and AMELY genes and their differential expression in males and females marked a significant milestone, leading to the development of practical methodologies for forensic investigations [1,2].

Thus, the method for determining sex consists of extracting DNA from tooth enamel and identifying the presence of one of the AMEL genes or both. Tooth enamel is a very reliable source of DNA as it is better preserved and less subject to degradation than other tissues such as bone, hair, or skin. The robust nature of enamel means that it can protect the embedded DNA from environmental insults, which often degrade other biological materials. This preservation advantage is particularly crucial in forensic scenarios where the condition of biological samples can be highly compromised due to various factors like exposure to elements, time, or trauma [3,5,6]. This simple approach is beneficial in identifying the biological sex of individuals of any age. It can be helpful in forensic situations where DNA may be of poor quality or DNA extraction is impossible. In highly decomposed or skeletonized remains, teeth often remain the best-preserved part of the body, making them a prime candidate for DNA extraction and subsequent analysis. The ability to determine sex from tooth enamel thus provides forensic scientists with a valuable tool in reconstructing biological profiles of unidentified individuals, aiding in criminal investigations, disaster victim identification, and archaeological studies [3,7,8].

Forensic scientists utilize various methodological approaches to isolate and analyze DNA from the whole tooth or tooth tissues. These methods often involve careful decontamination of the tooth surface to prevent contamination, followed by specialized techniques that allow for the extraction of the trapped DNA. Advanced molecular methods, such as polymerase chain reaction (PCR) and next-generation sequencing (NGS), are then employed to amplify and identify the specific AMEL genes. Developing these sophisticated methodologies has significantly enhanced the accuracy and reliability of sex determination from tooth enamel, making it a standard practice in forensic science [3,7].

The ability to extract and analyze DNA from tooth enamel has transformed the field of forensic anthropology, enabling scientists to reconstruct biological profiles with greater precision. For instance, in cases where skeletal remains are fragmented or incomplete, sex identification from tooth enamel can provide critical information for constructing a biological profile. This information, combined with other forensic evidence, such as age estimation and pathological markers, enhances the accuracy of victim identification and contributes to solving criminal cases [3,6,7].

Despite the advancements in amelogenin-based research, several challenges and limitations persist. One of the primary challenges is the potential for contamination during DNA extraction and analysis. Given the sensitivity of molecular techniques, even small amounts of contaminant DNA can compromise the accuracy of the results. Therefore, stringent protocols and contamination control measures are essential to ensure the reliability of the findings. Additionally, variations in the quality and preservation of enamel across different samples can impact the efficiency of DNA extraction and subsequent analysis, necessitating the development of standardized procedures to account for these differences [3].

Another problem that might interfere with sex identification through PCR techniques is the allelic dropout phenomenon. This was first described by Navidi and Arnheim [9], and it is known to reduce the efficiency of PCR-based identification [10]. This concerns the complete or partial inability to amplify one allele due to single nucleotide variants in the oligo primer binding site. This variant is usually close to the 3′ end of the primer binding site [11]. This limitation has been analyzed when using Y-STR in sex determination [12] and when using amelogenin for the same purpose [13]. So, this can lead to misinterpretation of the amelogenin use in sex identification. Several mutations that can lead to allelic dropout have been identified in the amelogenin gene [14,15]. As a result of this phenomenon, it is only possible to unambiguously identify the male sex through this methodology.

Future research in amelogenin-based sex determination should address these challenges and further refine the methodologies to enhance their accuracy and reliability. This includes the development of more robust extraction techniques, improvements in contamination control, and the standardization of protocols across laboratories. Additionally, expanding the scope of research to include diverse populations and environmental conditions will provide a more comprehensive understanding of the factors influencing the preservation and analysis of amelogenins in enamel [3].

The implications of amelogenin-based sex determination extend beyond forensic science into archaeology and anthropology. In archaeological contexts, determining the sex of individuals from ancient remains can provide insights into past societies, including social structures, gender roles, and population dynamics. For example, sex determination from enamel proteins can help identify burial patterns, revealing whether certain burial sites were reserved for specific genders or if there were differences in burial practices based on sex. This information contributes to a more nuanced understanding of ancient cultures and their social organization [8].

In addition to sex determination, preserving amelogenins in enamel also holds promise for other genetic studies. For instance, researchers can investigate genetic diversity and population structure by analyzing variations in amelogenin genes across different populations. This approach can shed light on the genetic relationships between ancient populations and their modern descendants, providing a genetic link that spans thousands of years. Such studies could enhance our understanding of human evolution and migration, offering a genetic perspective on historical events and population movements [8].

This scoping review aims to explore systematically and map the existing scientific literature regarding the use of amelogenin proteins, particularly their coding genes, AMELX and AMELY, in determining biological sex from tooth enamel. It will assess the methodological approaches, evaluate the efficacy of these proteins as biomarkers in forensic science, and identify any existing gaps in the research. By providing a comprehensive overview of the current knowledge, this review will highlight the strengths and limitations of using amelogenin-based sex determination and suggest areas for future research.

The review will also consider the implications of the preservation properties of dental enamel and its proteins for genetic analysis, focusing on their applications in archaeological and contemporary forensic contexts. The remarkable preservation of enamel proteins over millennia opens exciting possibilities for genetic studies of ancient populations, allowing researchers to explore questions related to human evolution, migration patterns, and population genetics. In contemporary forensic contexts, the resilience of enamel to degradation

ensures that it remains a reliable source of DNA for identifying individuals in challenging scenarios, such as mass disasters or crime scenes with limited biological evidence.

2. Materials and Methods

This research protocol was drawn up according to the Joanna Briggs Institute (JBI) model [16–18], which leads to the formulation of the following question: Do molecular methods allow for the determination of sexual dysmorphism for forensic identification? Thus, the acronym PCC will stand for population (P): molecular methods; concept (C): determination of sexual dysmorphism; and context (C): forensic identification.

The items identified in the reports prepared for the guidance of systematic reviews and the extension of meta-analyses (PRISMA-ScR) will be used for the final evaluation. This protocol was registered in the OSF (https://osf.io/3jnmc/) (accessed on 14 May 2024)).

2.1. Inclusion and Exclusion Criteria

This review will include articles that utilize molecular methods in forensic dentistry, focusing on the relationship between forensic dentistry and sex determination through the identification of amelogenin. Eligible studies must use teeth as samples and may include in vitro studies published in Portuguese, English, French, or Spanish without temporal restrictions. Excluded from this review are articles that do not use amelogenin for determining sex in the unidentifiable corpse, as well as those employing other methodologies such as orthopantomography, cone beam computed tomography (CBCT), tooth wear, oral pathology, crime scene investigations, odontometry, and analyses involving alternative samples like blood, bone, muscles, or buccal swabs. Review articles and meta-analyses are also excluded to maintain a focus on primary research studies.

2.2. Search Strategy

Two reviewers developed the search strategy, which underwent peer review via a third expert reviewer using the Peer Review of Electronic Search Strategies (PRESS) checklist [19]. For this scoping review, the search will be conducted across PubMed, MEDLINE (via BVS), and CINAHL (via EBSCO host), implementing the research strategy recommended by the Joanna Briggs Institute (JBI).

On 14 April 2024, a preliminary search in MEDLINE (via BVS) and CINAHL (via EBSCO host) was performed to determine the keywords and index terms relevant to the topic. This facilitated the search strategy for each database, detailed in Table 1. Following the search, the identified articles will be compiled in the ENDNOTE X9 version, and the RAYYAN tool will be utilized to eliminate duplicates. Additionally, the reference lists of all sourced articles will be examined to include further studies.

2.3. Study Selection

Two reviewers will independently collect data from the selected articles to determine their inclusion in this scoping review. Any doubts and disagreements will be resolved through discussion with a third reviewer, following the Peer Review of Electronic Search Strategies (PRESS) checklist [19].

Initially, two independent reviewers will check the titles and abstracts of articles, followed by a full-text review. To establish at least a 75% consensus between the reviewers, 5% of the total articles will be preliminarily analyzed. Subsequently, 2% of the full-text articles will be further evaluated to maintain this level of agreement.

The studies that align with the main objectives of this review will be selected through a comprehensive process that includes the identification, selection, eligibility assessment, and application of predefined inclusion and exclusion criteria along with research limiters. This careful process ensures the thoroughness and reliability of the review. The data extracted from each selected article will detail the sample size, methodology, study method, and primary evidence pertinent to the objectives of this review, as outlined in Table 2.

Table 1. Bibliographic research strategy.

Database	Articulation of Keywords	Number of Articles
PubMed	("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)	("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)	("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

The selection process is documented in a PRISMA-ScR flow diagram [20] (Figure 1).

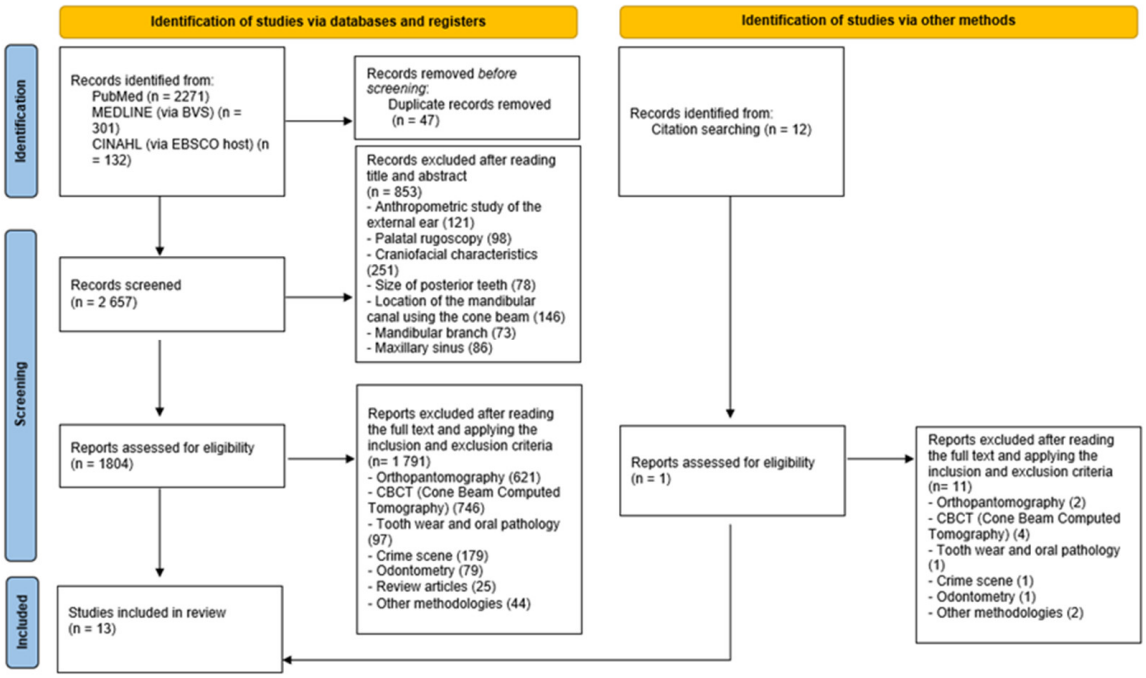

Figure 1. The flowchart of the article's selection process was adapted from the PRISMA 2000 flow diagram [20].

Table 2. Form used for data collection.

Scoping Review Title	Sexual dimorphism identification through molecular methods: Protocol of a Scoping Review.
Review objective(s)	Map the scientific evidence related to identification of sexual dimorphism using molecular methods on the detection of amelogenin gene.
Review Question(s)	Do molecular methods allow the determination of sexual dimorphism for forensic identification?
Inclusion/Exclusion Criteria:	
Population	This review will select studies using human teeth.
Context	This review will consider studies using molecular methods.
Concept	This review will consider studies that address the identification of amelogenin gene through molecular methods.
Types of Evidence Sources	This scoping review will consider any in vitro quantitative, qualitative, and mixed methods study designs for inclusion.
Evidence Source Details and Characteristics	
Author(s)	
Year of Publication	
Origin/Country of Origin (where the source was published or conducted)	
Aims/Purpose	
Population and Sample size	
Details/Results extracted from the Source of Evidente	

2.4. Data Analysis and Presentation

Upon completing the complete article assessments included in this review, data extraction will be aligned with the study's objectives and research questions using a methodology endorsed by the Joanna Briggs Institute [16–18]. The extracted data will include the article's title, author(s), publication year, country of origin, study type, objectives, and results, as summarized in Table 2.

The data presentation will be narrative, employing a qualitative assessment tool and associated coding strategies to thoroughly address the aspects of sexual dimorphism determination. Additionally, a detailed table will present this data and other relevant information to effectively map the available evidence and enhance the content provided in each article. This approach will facilitate the identifying, characterizing, and synthesizing of knowledge pertinent to this review's aims and objectives.

3. Discussion

This scoping review is of utmost importance as it aims to synthesize the existing literature on using amelogenin in forensic dentistry, particularly for sex determination. This review addresses a fundamental aspect of forensic science that directly affects legal and investigative processes by mapping the current evidence base and identifying research gaps. Focusing on a broad selection of articles and studies will also provide a comprehensive overview of the methodologies, outcomes, and regional variations in applying amelogenin for forensic purposes.

The findings of this review are expected to have a tangible impact on forensic practices and protocols. Given the reliance on amelogenin for sex determination in forensic settings, understanding the range of techniques and their efficacy can help standardize procedures, thus significantly improving the accuracy and reliability of forensic identifications. Moreover, the results could influence policy, particularly in standardizing forensic practices

across different jurisdictions. Establishing uniform protocols for amelogenin-based sex determination could enhance cross-border cooperation in criminal investigations and disaster victim identification, ensuring that forensic evidence is reliable and admissible in various legal systems.

Preliminary searches have revealed variability in the methods and applications of amelogenin-based sex determination. This review will highlight these areas needing more robust research and provide a clear roadmap for future studies. For instance, it will focus on the efficacy of amelogenin in degraded samples and its application in mass disaster scenarios. Identifying these gaps will guide future primary research and encourage the development of advanced methodologies or alternative biomarkers. Specifically, the review will consider the effectiveness of amelogenin in conditions where other biological materials may be compromised, such as in cases of extreme environmental exposure or prolonged decomposition.

This review will employ a comprehensive search strategy across multiple databases to ensure a wide capture of relevant studies. However, the exclusion of non-English articles might limit the scope. The chosen methodological framework, endorsed by the Joanna Briggs Institute, provides a structured approach to scoping reviews. This framework ensures a rigorous and systematic examination of the literature, enhancing the reliability and reproducibility of the review's findings.

The scope of this review extends beyond simply cataloguing existing studies; it also aims to evaluate the methodologies employed in these studies critically. By assessing the strengths and weaknesses of different approaches to amelogenin-based sex determination, the review will identify best practices and recommend standardized protocols that can be adopted globally. This critical appraisal will examine the technological advancements in DNA extraction and amplification techniques and the impact of sample preservation and contamination control measures on the accuracy of forensic results.

Furthermore, it will contribute to the theoretical understanding of forensic identification techniques by mapping the use of amelogenin in forensic dentistry. It will explore the biological underpinnings of amelogenin as a sex determinant and its practical application in forensic science, potentially influencing future theoretical advancements in forensic methodology. Understanding the molecular basis of amelogenin's role in enamel formation and its genetic coding will provide insights into its stability and reliability as a forensic marker. This biological perspective will be integrated with practical considerations, such as the feasibility of implementing amelogenin-based techniques in routine forensic practice and their cost-effectiveness compared to other methods.

In addition to its theoretical contributions, it will have practical implications for forensic practitioners. By compiling and analyzing data on the use of amelogenin in various forensic contexts, the review will offer practical recommendations for its application in different investigative scenarios. For example, it will provide guidelines for selecting the most appropriate DNA extraction methods for various types of enamel samples, considering factors such as sample age, preservation conditions, and available forensic resources. These guidelines will be invaluable for forensic laboratories seeking to implement or refine amelogenin-based sex determination protocols.

Moreover, it will address the implications of amelogenin research for forensic science education and training. By highlighting the importance of this protein in forensic investigations, the evaluation will underscore the need for specialized training in amelogenin-based techniques for forensic professionals. Educational programs and training workshops can be developed to disseminate best practices and ensure forensic practitioners have the knowledge and skills to utilize amelogenin effectively.

However, this protocol acknowledges several potential limitations that may impact the scoping review on the use of amelogenin in forensic odontology for identification: the availability of relevant literature on this topic may be limited, and existing studies may vary significantly in quality and scope; there is a potential for publication bias, where studies with positive results are more likely to be published, potentially skewing the review's

findings; this review will primarily include studies published in Portuguese, English, French, or Spanish, which may exclude relevant research published in other languages; it will focus on studies published within a specific time frame, possibly excluding older studies that could still be relevant or newer studies that have not yet been published; significant heterogeneity in the methodologies and outcomes of included studies may pose challenges in synthesizing the findings; the process of data extraction and interpretation is subject to subjective biases; rapid advancements in forensic technology may make some reviewed studies outdated or less relevant to current practices; variations in regulatory and ethical standards across different countries could affect the applicability and generalizability of the review's findings. The final scoping review will address these limitations to ensure a comprehensive and balanced analysis.

The implications of this review extend to interdisciplinary research as well. Integrating forensic science with molecular biology, genetics, and bioinformatics will be essential for advancing amelogenin research. Collaborative efforts between these disciplines can lead to the development of more sophisticated analytical tools and techniques, enhancing the precision and reliability of sex determination from enamel samples. For instance, applying bioinformatics tools to analyze genetic variations in amelogenin genes across different populations could provide deeper insights into the evolutionary and demographic patterns that influence its forensic applicability.

Furthermore, it will consider the ethical implications of using amelogenin-based sex determination in forensic investigations. The accuracy and reliability of forensic evidence are paramount for ensuring justice, and the potential for misidentification due to methodological errors or contamination must be carefully addressed. By promoting rigorous standards and ethical practices in amelogenin research and application, the review will contribute to the integrity and credibility of forensic science.

This review will significantly enhance our understanding of this critical forensic technique by mapping the current evidence base, identifying research gaps, and offering practical recommendations. The anticipated impact of the review on forensic practices, policies, and education underscores its importance for advancing forensic science and improving the accuracy and reliability of forensic identifications. The review will pave the way for future research and innovation in amelogenin-based forensic methodologies through its thorough and systematic approach.

4. Conclusions

This review will be a starting point for mapping the available scientific evidence on the subject under study. It will contribute to the evaluation and knowledge of the importance of amelogenin in sex identification and the importance of teeth in preserving DNA in human remains in forensic studies.

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