Alteration of the AMELX gene in amelogenesis imperfecta. A brief review

Omar Tremillo-Maldonado,1 Nelly Molina-Frechero,1 Rogelio González-González2 and Ronell Bologna-Molina3

1Health Care Department, Universidad Autónoma Metropolitana Unidad Xochimilco, Ciudad de México, Mexico; 2Universidad Juárez del Estado de Durango, Faculty of Dentistry, Department of Research, Durango, Mexico; 3Universidad de la República, School of Dentistry, Area of Molecular Pathology, Montevideo, Uruguay

Abstract

Amelogenesis imperfecta is a group of developmental disorders of the dental enamel that is mainly associated with mutations in the AMELX gene. Clinically, it presents different phenotypes that affect the structure and function of dental enamel both in primary and secondary dentition. The purpose of this study was to conduct a literature review on the AMELX functions and mutations that are related to amelogenesis imperfecta. A literature search was carried out in two databases: PubMed and Web of Science, using the keywords “AMELX”, “amelogenin”, “amelogenesis imperfecta” and “AMELX mutation”. Forty articles were reviewed, with AMELX being found to be the predominant gene in the development of dental enamel and amelogenesis imperfecta by altering the structure of amelogenin. In the past few years, the characteristics of the amelogenesis imperfecta process have been described with different phenotypes of hypoplastic or hypo-mineralized enamel, and different mutations have been reported, by means of which the gene sequencing and the position of mutations have been determined.

KEY WORDS: AMELX. Amelogenin. Amelogenesis imperfecta. AMELX Mutation.

Introduction

Dental enamel is a highly mineralized tissue. Its formation, also known as amelogenesis, is a process that begins when enamel-forming ameloblast cells deposit a thin layer of protein-rich enamel matrix such as amelogenin and, to a lesser extent, enamelin, ameloblastin and tuftelin, whose function is to control the process of crystal growth and enamel mineralization; these proteins are degraded by ameloblast-secreted proteinases. This process ends with enamel crystals’ mineralization, elimination of proteins and maturation, giving rise to a highly mineralized tissue.

Amelogenin is the predominant protein of the enamel matrix, and its alteration clinically manifests as amelogenesis imperfecta.1,2

Amelogenesis imperfecta is a hereditary genetic disease that comprises a group of enamel malformations with different developmental disorders that affect tissue formation, mineralization and thickness, which can exhibit hypoplasia and hypo-mineralization, and the tooth can thus acquire a yellowish color, with rough texture and loss of enamel translucency, as well as dental function.1,3,4

Kim et al.5 express that hypoplasia in the enamel phenotype is caused by a developmental deficiency during the secretion of the enamel matrix and that hypo-mineralization clinical manifestations may be due to disorders during the matrix maturation. There are different clinical manifestations of amelogenesis imperfecta, which were described in 1988 by Witkop, who classified enamel development alterations into four main groups according to the enamel structure,
with or without predominance of hypoplastic or hypo-mineralized areas.6

The prevalence of amelogenesis imperfecta has a global mean of less than 0.5 %; i.e., less than one patient in 200.7 In different populations, the reports have shown differences in the prevalence of amelogenesis imperfecta. Epidemiological studies show ranges of 43:10,000 in Turkey,8 14:10,000 in Sweden,9 10:10,000 in Argentina,10 1:14,000 in North America4 and 1.25:10,000 in Israel.11 In studies of dental abnormalities, the prevalence of amelogenesis imperfecta was three in 1123 patients from India,12 0.08 % in 1200 patients from Turkey,13 two patients in 860 Mexican patients14 and four in 478 patients in Brazil.15

Currently, the understanding of amelogenesis imperfecta is important, as well as the role of enamel formation genes and proteins and their genetic variations. AMELX is one of the main genes that participate in the formation of the enamel matrix, as well as in the signaling required by ameloblasts to fulfill its functions during enamel development. Based on this, the purpose of this work was to conduct a review of the scientific literature aiming to understand the role of AMELX in the process of tooth enamel development, as well as the importance of its alterations in the development of amelogenesis imperfecta.

Method

A descriptive study was carried out through a search of research and review articles published up to 2016. The search was conducted in Pub-Med and Web of Science using the keywords “AMELX”, “amelogenin”, “amelogenesis imperfecta” and “AMELX mutation”.

Seventy-four articles were found from the year 1984 to 2016, all in English, which met the following criteria:
- Inclusion criteria: experimental and clinical trials of AMELX and amelogenin, studies of mutations in families or populations with amelogenesis imperfecta and literature reviews on AMELX, amelogenin and mutations.
- Exclusion criteria: articles that include other ameloblast-derived proteins, such as enamelin and ameloblastin, which occur in minimal proportions; articles that study or describe AMELX functions unrelated to amelogenesis imperfecta; studies on amelogenesis imperfecta related to other genes, other alterations and medications.

Figure 1 shows how the exclusion of 34 articles was carried out.

The bibliographic search was divided into two periods: 1984-2008 and 2011-2016. A selection was made of those AMELX gene variations regarded as pathogenic by the National Center for Biotechnological Information.16

Results

Articles from 1984 to 2016 were found, which were organized in the two indicated periods. Works of the first period (1984-2008) were focused on the sequencing and functions of the gene and protein in the amelogenesis process; the first study on mutation of the gene dates from 2007 (Table 1).

No articles with the selected criteria were found in 2009 and 2010; as of 2011, multiple studies of the AMELX gene have been carried out (Table 2). Tables 1 and 2 show the most important discoveries related to AMELX and amelogenin over time; as a
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result of that review, the information referred to below was obtained.

Study of the AMELX gene

AMEL is a gene found in sex chromosomes X and Y. According to the chromosome it belongs to, it is called AMELX and AMELY. Sex chromosome X AMEL is located in the p22.3-p22.1 region and is formed by more than 8000 base pairs. AMEL has been identified in different organisms since the first amphibians.

In 2008, Kawasaki associated AMELX with the family of genes that encode for secretory calcium-binding phosphoproteins (SCPP). The SCPP family of genes includes 21 genes that encode proline and glutamine-rich acidic proteins clustered on chromosome 4, with the exception of AMEL, which is located on sex chromosomes X and Y. SCPP genes are present in humans, mammals and other animal species; this family of genes participates in the formation of calcified tissues, such as bone and teeth.

In 2007, Iwase et al. reported that AMELX is nested in intron 1 of the ARHGAP6 gene; by means of a study in different mammals and amphibians, they found that AMELX is found within said gene, which suggests that this location has been maintained throughout history since the first amphibians.

Prakash et al., by inactivating the ARHGAP6 gene in a study in mice, reported that suppression of said gene causes different enamel phenotypes and abnormalities in other tissues or behavioral anomalies, suggesting that ARHGAP is not essential in the

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<tr>
<td>Takagi et al.26</td>
<td>Bovine tissue</td>
<td>Japan</td>
<td>1984</td>
<td>– First study to sequence bovine amelogenin amino acids.</td>
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<td>Lau et al.17</td>
<td>Human and mouse tissue</td>
<td>United States</td>
<td>1989</td>
<td>– First study in humans to locate AMEL in chromosome X p22.1-p22.3 region and in chromosome Y peri-centric region. – In mouse, only in chromosome X.</td>
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<td>Salido et al.20</td>
<td>Human tissue</td>
<td>United States</td>
<td>1992</td>
<td>– Y-derived amelogenin accounts for only 10% of amelogenesis</td>
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<tr>
<td>Iwase et al.22</td>
<td>Patients</td>
<td>France</td>
<td>2007</td>
<td>– Reports AMELX mutation with different hypoplastic or hypo-mineralized enamel phenotypes. – Exon 6 was the most variable region.</td>
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<td>Kawasaki K. et al.25</td>
<td>Literature review</td>
<td>United States</td>
<td>2008</td>
<td>– Reports that AMELX belongs to the secretory calcium-binding phosphoprotein (SCPP) gene family.</td>
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<td>Lee et al.45</td>
<td>Patients with AI</td>
<td>Korea</td>
<td>2011</td>
<td>– Reports AMELX gene new mutation with hypo-mineralized phenotype even in unerupted teeth. – In 7-exon AMELX, translation starts in exon 2 and exon 7 encodes a single amino acid.</td>
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<td>Hu et al.24</td>
<td>Patients with AI</td>
<td>United States</td>
<td>2012</td>
<td>– Arghap6 gene suppression, with exception of intron 1, showed no enamel changes.</td>
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<td>Fukuda et al.27</td>
<td>Mouse animal tissue</td>
<td>Japan</td>
<td>2013</td>
<td>– Reports AMELX interaction with bone tissue-forming genes.</td>
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<td>Jacques et al.29</td>
<td>Animal model with mice</td>
<td>France</td>
<td>2014</td>
<td>– AMELX participation in enamel matrix and maxillary teeth growth.</td>
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<td>Cho et al.3</td>
<td>Patients with AI</td>
<td>Korea</td>
<td>2014</td>
<td>– Reports the production of AMELX gene alternative proteins, although their functions are unclear.</td>
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<td>Guo et al.37</td>
<td>Mouse animal tissue</td>
<td>United States</td>
<td>2015</td>
<td>– Reports that amelogenin deficiency favors the presence of acidic zones in developing enamel, which affects its maturation.</td>
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<tr>
<td>Le et al.40</td>
<td>Mouse animal tissue</td>
<td>United States</td>
<td>2016</td>
<td>– Reports that AMELX exon 4 participates in amelogenin formation in addition to participating in bone tissue formation.</td>
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formation of tooth enamel. When both these genes were studied in a population of two families with amelogenesis imperfecta, Hu et al. reported in 2012 that AMELX is the main gene that participates in amelogenesis imperfecta. They demonstrated that ARHGAP6 has no relationship with the formation of enamel or bone tissue.

**Amelogenin**

In humans, AMELX encodes the amelogenin protein through seven exons, with translation starting in exon 2 and ending in exon 7, which only encodes for one amino acid. Amelogenin is a secretory calcium-binding phosphoprotein, whose sequence involves 191 amino acids. This protein can be encoded by AMELX and AMELY; however, Salido et al. found that AMELY accounts only for 10% of amelogenesis, and that AMELX is therefore the main amelogenin-forming gene in that process. In a study on the Y chromosome, Jobling et al. reported that AMELY has little participation in enamel formation and no expression in other tissues. Furthermore, other authors were able to establish that AMELY-formed amelogenin fails to cover the deficiencies in enamel development caused by AMELX alterations, and hence they consider that further studies should be conducted to determine the participation of AMELY-generated amelogenin.

Amelogenin is the most abundant protein in the enamel formation process, since it comprises 90% of enamel matrix. There are different variants of this protein known as isoforms, which result from alternative splicing during the transcription and translation process of the protein; these variations can range from different lengths to significant changes in the structure of the protein. Currently, it is not entirely known whether there are functional differences in the protein different isoforms. For example, amelogenin M180, which is the most common isoform, generates an enamel that is more resistant to erosion and more fragile to fracture in comparison with normal enamel. In studies carried out in mice, identifying the protein modification was possible and, hence, the changes in enamel phenotypes. These variations have been associated with disorders of the enamel structure and amelogenesis imperfecta but, on the other hand, the existence of these amelogenin variants in other animal species has been suggested to be driven by biomechanical demands exerted on enamel.

In 2009, Delak et al., based on bioinformatics, created a porcine amelogenin simulated three-dimensional model. They found that the amelogenin structure is part of a group of proteins that has the ability to maintain an unfolded or contracted form because it performs different functions with each form. Figure 2 shows a graphical simulation of unfolded porcine amelogenin, with the terminal groups being indicated. Amelogenin fulfills functions such as inducing apatite crystal formation and controlling its shape and growth; it protects crystals and ameloblasts by controlling pH changes. In addition, it participates in signaling with osteoblasts in the process of bone tissue formation.

Enamel crystals growth occurs with the mineralization process; by means of nanospheres, amelogenin guides crystals longitudinal growth, preventing them from binding to each other. When this process is developed, there is gradual elimination of the enamel matrix, mainly constituted of amelogenin, and the crystals begin to grow in width, ending up laterally bound. Guo et al. reported that amelogenin plays an important role in pH control, since when there is...
In a 2010 case report, Lindermeyer et al. associated amelogenesis imperfecta genotype and phenotype. They reported that amelogenin correct elimination is essential to the development of healthy enamel, and it is therefore important for this protein not to be mutated or in incorrect amounts. Currently, amelogenesis imperfecta studies have focused on identifying mutations that alter the synthesis of the amelogenin protein, which, when exhibiting different characteristics, is not recognized by the proteinases responsible for its elimination. Due to this lack of identification, amelogenin remains in excess, which translates into a hypo-mineralized enamel at the end of its formation.

Due to the importance of AMELX in the development of dental enamel, mutations of this gene cause variations in the protein, which affects the development of enamel. Currently, 16 AMELX mutations are known to modify amelogenin and cause amelogenesis imperfecta with different characteristics of enamel hypo-mineralization and hypoplasia that are not related to any other disease. Disturbances in the development and organization of crystals, as well as in amelogenin signaling and protection functions may cause different amelogenesis imperfecta phenotypes, with differences in severity according to the location of AMELX mutations.

Hu et al. carried out a review of the 16 mutations described in the literature and presented a graphic representation of the AMELX gene, the seven exons that make it up, and the exon areas that encode the protein; in addition, they located the exons where each mutation occurs (Figure 3).

The studies of AMELX gene mutations are important since there are genotypic variations in different populations; the identification of mutations in different human groups will impact on the understanding of AMELX and amelogenesis imperfecta.

Wright et al. studied 463 subjects from 54 families with some variety of amelogenesis imperfecta, in whom they sequenced different genes; they found mutations in the AMELX gene that can be linked to different visible degrees of enamel hypo-mineralization and hypoplasia, ranging from small pits in the enamel and grooves, to thinning or lack of tissue. They
reported three different AMELX mutations associated with amelogenesis imperfecta in said population.46

Other investigations found the already reported mutations in 40 % of families and 60 % of individuals with amelogenesis imperfecta; in the rest, identifying them was not possible, which suggests that there are still unstudied mutations or non-coding regions of genes involved in the development of this disease, in addition to the possibility of new genes not included in amelogenesis imperfecta etiology.46,47

Mutations in AMELX contribute to amelogenin structure alteration, which can affect the formation of enamel through different routes, such as an alteration of the protein functions, in addition to its interaction with ameloblasts and other important proteins in the development of this tissue. AMELX main variations are c.152C > T, c.571G > T, c.208C > A, c.2T > C, c.11G > single nucleotide C and c.155delC, c.14–22delTTTTTATGG, c. 473delC and c.541delC, which are mutations by deletion of different nucleotides that result in amelogenesis imperfecta with dominant hypoplasia and hypomineralization linked to chromosome X.16,48,49

As a result of this review, we can conclude that AMELX is the most predominant gene in the development of enamel and amelogenesis imperfecta. Amelogenin induces apatite crystal formation and controls pH and signaling with bone tissue-forming cells surrounding the tooth; AMELX mutations produce alterations in this protein functions, such as lack of cell-enamel matrix interaction, pH changes, enamel crystals abnormal growth and, subsequently, amelogenin interaction with proteins. In addition, the position of mutations in the gene sequence of has been found to generate different amelogenesis imperfecta phenotypes, with clinical features of hypo-mineralized or hypoplastic enamel.

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References