# Genetic and Epigenetic Aspects of Amelogenesis Imperfecta and Dentinogenesis Imperfecta

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**Abstract.** Amelogenesis imperfecta (AI) and dentinogenesis imperfecta (DGI) are hereditary dental disorders. AI is a developmental condition resulting in enamel defects of all or almost all teeth. The prevalence ranges from 1:700 to 1:14,000 for AI and from 1:6000 to 1:8000 for DGI. DGI is a collective of autosomal dominant conditions of anomalous dentine structures affecting either the primary or both the deciduous and permanent dentitions. AI is caused by disturbed developmental processes, such as mutations of the AMELX gene that encodes secretion of extracellular matrix proteins from ameloblasts during enamel formation. AI can also arise due to mutations in several other genes that encode proteins with a role in amelogenesis. DGI results from mutations in the gene encoding dentine sialophosphoprotein (*DSPP*), COL1A1 and COL1A2. In addition, both AI and DGI can be promoted by interference of the regulatory functions of key genes through influence by environmental factors such as trauma, chemicals or systemic diseases, and epigenetic factors such as DNA methylation. This paper aims to review the genetic and epigenetic etiological factors of AI and DGI.

**Keywords:** Amelogenesis imperfecta · Dentinogenesis imperfecta · Genetic · Epigenetic

## 1 Introduction

Amelogenesis imperfecta (AI) and dentinogenesis imperfecta (DGI) are hereditary dental diseases. AI is characterized by developmental enamel defects. The enamel might be hypoplastic, hypomineralised or both and teeth influenced might be stained, soft and inclined to breaking down [1, 2]. AI is divided into four types namely hypoplastic, hypomineralized, hypomaturation and hypomaturation–hypoplastic with taurodontism. In hypoplastic AI, the teeth look small, and the clinical areas of the crown contain very thin or nonexistent enamel. Enamel from hypomineralized AI is opaque and yellow or


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light brown in color. In hypomaturation type, the teeth appear normal in enamel thickness but show low values of radiodensity and mineral content. In the last type of AI is hypomaturation-hypoplastic with taurodontism, the teeth appear mottled with yellow color and enlarged pulp chambers in radiographs [3, 5, 6].

DGI has the clinical characteristics of discolored teeth and defective tooth structure in the form of a bulbous crown. Radiographs show pulp chambers that appear small. Disruption in the mineralization process results in shearing enamel and leaves weak dentin exposed underneath. DGI is divided into three types, of which type I occurs in patients with osteogenesis imperfecta. The teeth arebrownish-yellow on both dentitions, translucent with notable attrition. Type II is known as transparent hereditary type without osteogenesis imperfecta, and is autosomal dominant. The appearance of the teeth of this type is similar with type I. In type III, known as brandywine type, the name comes from a tri-racial population from Maryland and Washington DC where this type was found. In this type, the manifestation can vary and show features like in DGI types I and II [15].

Mutations in AMELX, ENAM, MMP20 and FAM83H genes can impact AI. AMELX, ENAM, and MMP20 are the genes that encode for the proteins in the development of teeth. These proteins promote formation of hard enamel, rich of calcium, to develop the protective top layer for teeth [6]. Mutations of the genes affect protein structure or inhibit the proper development of protein. Then the enamel becomes thinner or soft, with yellowish or brown color. The teeth with enamel defects are weak and easily broken [4, 6]. About half of such gene mutation cases happen due to mutations of FAM83H [4].

Besides being dictated by various genes, the formation of enamel and dentine is also influenced by epigenetic factors. Epigenetics refers to the study of innate changes in organisms caused by modification of gene expression. Modification of gene expression is a natural process that occurs in cells to adjust the type and amount of proteins expressed in cells. DNA methylation and histone modification are two major epigenetic modifications of gene expression. In DNA methylation, a methyl group is added to mark DNA, for activating or suppressing the DNA expression. In histone modification, epigenetic factors bind to the tail of the histones, changing the level of DNA wrapped around the nucleosomes. Histones are a type of proteins around which DNA can bind during chromatin formation. The level of DNA wrapped around histones changes gene expression [10, 13].

It is important to understand the genetic and epigenetic mechanisms of diseases in order to overcome or even prevent them. In this paper we aim to discuss the genetic and epigenetic mechanisms involved in the occurrence of amelogenesis and dentinogenesis imperfecta.

## 2 Amelogenesis imperfecta

Amelogenesis imperfecta (AI) is a enamel defect that is associated with genetic alteration. It can affect the structure and appearance of whole or practically all the teeth, with varying severity, and could be linked to altered morphological or biochemical features [1]. AI can also occur during tooth developmental stages. The characteristic of AI is hypoplasia and/or hypomineralisation that can show autosomal dominant, autosomal recessive, and sex-linked conditions [2]. Those conditions affect the structure and

appearance of the enamel, such as enamel thickness, discolouration, staining, sensitivity, fragility, and rapid wear [2, 3]. The clinical appearance will vary individually, and can be impacted in both primary and secondary dentition [3].

A study found 14 types of AI. The classification is divided by type of mutation, location and specific gene involved.3 Furthermore, AI can be associated with a syndrome elsewhere in the body [3, 4].

### 2.1 Aetiology

The enamel development is initiated by ameloblast cells through synthesis and extracellular matrix secretion [2, 5]. The process of tooth enamel development or amelogenesis has three stages: the secretory, transition and maturation phases. During the maturation phase, minerals are deposited on crystallites which growth in width and thickness to close with the closest crystal [2]. The dental enamel crystal ion is close to hydroxyapatite (HA). Protein is degraded by proteases, then the tissue fluid will replace it. The HA expands in size and thickness due to progressive hydrolysis of proteins [6].

Protein activity is mediated by genes encoding the enamel crystal deposition processes. There are three main proteins in the formation of enamel, namely amelogenin, enamelin and ameloblastin [3]. Secretion of enamel proteins along with proteases will develop a complex of enamel matrix. Increased proteolytic activity occurs during the transition phase. At the maturation stage, accumulated enamel proteins almost vanish from the matrix. Genetic defects of proteins or proteinases that cause changes in enzymatic protein degradation can cause pathological changes throughout the process of the genesis of the enamel [3, 7].

Mineralized tissue with over 95% of hydroxyapatite crystals forms the dental enamel [2, 6]. The formation of this structure is managed by ameloblasts through the interaction of a number of organic matrix molecules that include amelogenin (*AMELX*; Xp22.3-p22.1, AMELY) [2–4, 6]; enamelin (*ENAM*; 4q21) [2–4, 6], ameloblastin (*AMBN*; 4q21) [2, 3]; tuftelin (*TUFT1*; 1q21, OMIM *600087) [2]; amelotin (*AMELOTIN*; 4q13) [2]; dentine sialophosphoprotein (*DSPP*; 4q21.3, OMIM *125485) [2]; and enzymes such as kallikrein 4 (*KLK4*; 19q13.3–q13.4) [2, 6] and matrix metalloproteinase 20 (*MMP20*; 11q22.3–q23) [2–4, 6].

### 2.2 Genetic Mutations

Amelogenesis imperfecta can vary in its causative origins. It is based on the gene involved, such as X-linked, autosomal dominant or recessive. Moreover, many cases happen from mutations of gene *FAM83H* due to autosomal dominant condition [6]. In some of the cases, *ENAM* is also autosomal dominant. Furthermore, AI can be as an autosomal recessive disorder in cases of ENAM or MMP20 as well. The autosomal recessive condition requires two copies of the gene in the impacted cell. When parents bring one copy of the autosomal recessive gene, it will not show the disease symptoms [4, 6].

Approximately 5 percent of the cases of AI, inherited in X-related patterns, are caused by mutations in the AMELX gene. If the mutated gene causes interference located on the X chromosome, it can be said that the abnormality is related to the X-linked hereditary

pattern. X-linked hereditary patterns involve one of two sex chromosomes. So, men with X-linked AI experience a greater likelihood of having more severe dental abnormalities than women with this condition [4].

### 2.2.1 Amelogenin

Amelogenin is a protein product from AMELX Xq22 and AMELY Yp11 gene. The amelogenin protein is important to develop enamel with proper structure and thickness. Amelogenin is the largest protein in enamel, more than 90% of the total enamel protein [2, 6] of which ameloblastin is about 5%, and enamelin around 2% [7–9].

Amelogenin is secreted by ameloblasts as a 25-kDa protein nascent with high degree of polarity that is in line with the high of C hydrophilic telopeptide terminal. The telopeptide will proceed fastly by specific endoproteinase and/or carboxyproteinase to make molecule intermediate of 23 kDa amelogenin. The stable form of 20 kDa molecule of amelogenin is the temporary result of the amelogenin intermediate of 23 kDa or single division from amelogenin of 25 kDa [9]. The slow process of protein hydrolysis is indicated by the 20 kDa amelogenin accumulation. Extracellular and enzymatic degradation occurs in amelogenin. This process produces inadequate peptides that generate a framework for mineralization and formation of enamel crystals [3, 7, 8].

There are about 14 mutations occurring in amelogenin – 5 substitutions of nucleotide, 7 small deletions and 2 large deletions. The deletion of 5kb from 5 to 7 of exon in the amelogenin gene interferes with the overall role of amelogenin so that it will produce enamel of normal thickness but strongly discolored and less mineralized. Hypoplastic enamel can result from a 9bp deletion in exon 2 coding for the signal sequence that produces normally mineralized enamel but in reduced thickness [8, 9].

Hypoplastic and/or hypomineralisation AI can occur due to the presence of nucleotide C deletions in different codons which have an impact on the premature termination codon and loss of protein C-terminus. Symptoms of AI can vary among affected members of the same family. Gene mutations in the form of substitutions can also cause hypomaturation type of AI; two substitutions, C to A and A to T, are explained in exon 6 [3]. The transcription-active amelogenin gene is found on both human X and Y chromosomes. However, AI has not been found with mutations in amelogenin Y. Also, the Y chromosome only accounts for about 10% of the amelogenin transcript.

### 2.2.2 Ameloblastin

Ameloblast cells express ameloblastin, that is also known as AMELIN. Ameloblastin is the largest nonamelogenin enamel protein matrix which is a tooth-specific glycoprotein. This protein is found at the area of ameloblasts secretion [2, 3]. The location of ameloblastin gene is at chromosome 4, which is the usual location of the hypoplastic AI. Ameloblastin is fundamental to molecular adhesion of enamel genesis and has a critical responsibility in binding and retaining phenotypic differentiation from ameloblast secretion [10].

### 2.2.3 Enamelin

The biggest enamel extracellular matrix protein is called enamelin [3]. It is a product of the *ENAM* gene which is found in chromosome 4. Enamelin plays a key role in the growth of elongated enamel rods. The mutation of *ENAM* gene represents an autosomal dominant condition of hypoplastic AI. Mutated enamelin has been identified from a substitution of single base splice on the intron 7 site. Hypoplastic enamel in both primary and permanent teeth result in yellowish teeth and hypersensitivity to cold stimuli in the affected family. Hypoplastic AI will impact both primary and permanent dentition [2, 6].

### 2.2.4 Enamelisin (MMP-20)

The gene for matrix metallopeptidase 20 (*MMP-20*) is found in the chromosome 11. Enamelisin is associated with autosomal recessive pigmented hypomaturation of AI. Most proteinase is linked with the development process of matrix enamel proteins. A mutation that causes disappearing MMP 20 activity results in a severe phenotype where enamel hypoplasia occurs to cover the dentin, disorganizes the prism pattern, and disrupts the enamel morphology [3, 6, 7].

### 2.2.5 Kallikrein 4 (KLK-4)

The KLK 4 is active during tooth development, and secreted by dentine which produces odontoblasts and by enamel which develops ameloblasts. The maturation stage of enamel development will be disrupted if there is a loss of KLK 4 function, affecting the formation of enamel crystals. Enamel crystals will influence the final deposition of 15–20% of minerals [3, 7].

### 2.2.6 FAM83H

FAM83H is an intracellular protein that can be reacted in different parts of the body. In oral tissue, the biggest expression of ameloblasts occurs on FAM83H. In many cases, AI occurs due to the FAM83H gene. It is the expression from autosomal dominant which can be hypo calcification from AI which impact the thickness of normal enamel that can decrease the mineral content [4, 6].

## 2.3 Epigenetic Regulation in Enamel Development

### 2.3.1 Methylation of DNA

The process of DNA methylation occurs in chitosan when chitosan is followed by guanosine (CpG dinucleotide). Hypomethylation or the loss of methyl group on DNA will affect DNA activation. Accumulation of methylation involves in physiological processes of X chromosome inactivation, genetic imprinting, and the gene expression in a specific tissue. These are the main mechanisms in epigenetic control from gene expression and maintaining genomic integrity [10, 11].

### 2.3.2 Histone Modification

The lysine residue in histone will cause the acetylation process and methylation, where H3 lysine 4 methylation will activate gene expression while H3 lysine 9 methylation will silence the gene to form heterochromatin. The mechanism of CpG is related to gene silencing. It appears to involve a specific relationship between methylated DNA protein bonds, followed by silencing complex histone deacetylase [12]. By methylation of DNA coupled with methyl protein bonds and also protein complexes containing histone deacetylation, the structure of DNA turns compact and chromatin solidifies. Methylation of local cytosine from certain sequences can directly interfere with transcription factor bonds. Then, hypermethylation of the coding region will reduce gene transcription [10–12].

### 2.3.3 Factors Associated with DNA Methylation

#### 2.3.3.1. Genetic Polymorphism and DNA Methylation

A methyl group in DNA can be linked to specific alleles. For example, folate status and the effect of DNA methylation alteration related to a mutation of methylentetrahydrolate reductase (MTHFR). The polymorphism is related to various pathological conditions and vulnerabilities that can be suspected due to heredity or congenital factors of DNA methylation between healthy and diseased individuals [10, 11].

#### 2.3.3.2. Interaction of Nutrition and Gene Methylation

Research in nutrition has recently shown that there are a few nutrition roles in arrangement of genome machinery. Some of vitamins and micronutrients are substrates and cofactors that regulate DNA synthesis and / or repair and influence gene expression. Nutritional deficiencies affect the integrity of a gene and the changes in DNA methylation. For example, zinc deficiency can reduce the role of methyl groups from S-adenosylmethionine (SAM) and produce hypomethylation of genomic DNA and histone hypomethylation. Also, selenium deficiency decreases genomic DNA methylation. Hypermethylation of DNA in cells where lung cancer occurs is also associated with vitamin C deficiency. Alcohol consumption can decrease the content of vitamin B12 that is needed for DNA methylation and protein synthesis. The deficiency of vitamin B12 can trigger hypomethylation of DNA [13].

## 3 Dentinogenesis Imperfecta

Dentin is part of tooth located between enamel and pulp chamber. It consists of hydroxyapatite minerals (70%), organic components (20%), and water (10%). The organic material contains 85% of collagen type I, and the rest of it is non-collagen protein. This protein contains 50% dentin phosphoprotein [14].

DGI is a defect of tooth formation that decreases the dentin mineral contents such as the mineral hydroxyapatite. Moreover, it also increases the water content in dentin extracellular matrix and disturbs the structure of dentin. DGI occurs due to interference in the development at the histodifferentiation stage, affecting both primary and permanent dentition. DGI heredity isof autosomal dominant type, with incidence ratio from 1:6000 to 1:8000 [14, 15].

Associated genetic disorder during the dentine development is one of the causative factors in the aetiology of DGI. His is caused by a mutated gene coding for dentine sialophosphoprotein (DSPP).

DSPP is a non-collagen protein for dentine formation. It contains serine that has an important role in mineralization and can be phosphorylated. In case of an adverse mutation in the corresponding gene *DSPP*, the abnormal dentine phosphoproteinwould affect the clinical appearance of teeth due to grayish or yellow-brownish color of the dentition, and significantly impaired dentine mineralization. The decreased hydroxyapatite content in dentine can then lead to easy tooth fractures and severe rates of dentine attrition [14].

### 3.1 Aetiology

Mutations in coding protein genes cause hereditary dentinal defects during dentine development. In DGI type I, the dentinal defect is from osteogenesis imperfecta (MIM 166240), an autosomal dominant condition that occurs due to a mis-sense mutation. It impacts on coding for two collagen genes type II, COLIA1 dan COLIA2. In dentinogenesis type II (MIM 125490) and III (MIM 125420) the mutations occur in a gene that encodes dentine sialophophoprotein (*DSPP*) [16]. Located in 4q22.1, it has 5 exons of about 8343 bp. *DSPP* is also expressed in other tissues like in kidneys, salivary glands, bones and lungs, but the protein levels in these secondary locations are considerably lower than in dentine [17].

There are three types of protein developed by polypeptide translation: dentin sialoprotein (DSP), dentin glycoprotein (DGP) and dentin phosphoprotein (DPP) [18, 19]. Dentin phosphoprotein (DPP) is the most active protein to cleavage, thus that it is highly phosphorylated. Therefore, DPP is involved in the nucleation of hydroxyapatite crystals and controlling its growth. The DPP contains a lot of aspartic acid and phosphorus. In cleavaging, the DPP moves to the mineralized areas associated with the occurrance of type I collagen. On the other hand, DSP is a highly glycosylated proteoglycan that develops dimers via intermolecular disulfide bridges. DGP has four phosphorylated serine residues and one glycosylated asparagine. These protein functions are involved in the initiation and control of mineralized dentin. The DSPP encodes DPP that affects mutations on coding the DSP area. These can be mis-sense, non-sense or *splicing* mutations [20].

Research on the pathogenesis of amelogenesis imperfecta (AI) and dentinogenesis imperfecta (DGI) have characterized many details on the mechanisms of these hereditary disorders of dental enamel and dentine, respectively. The involved causative genetic, epigenetic and environmental factors have been more thoroughly studied for AI to reveal also the involved mechanisms, processes and interactions in the main types of AI.

For the main types of DGI, there appears to remain much scope for studies on genetic and epigenetic mechanisms and contributing factors. Both AI and DGI are disorders that deserve continued attention for mitigating their impact on the patient dentition, by developing further informed approaches to treat and perhaps even prevent these abnormalities.

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