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Burden of Hereditary Enamel Disorders

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Abstract:

Dental enamel protects against the invasion of bacterial pathogens deep into the innervated layers of the tooth. Hereditary enamel disorders referred to as amelogenesis imperfecta (AI) can severely affect the development and mineralization of dental enamel compromising these functions. This rare disorder is often visible, carry a significant psychological and financial burden, and co-segregates with disease in other organs. Pathological variants in over 100 genes affect the enamel formation. Here we describe the biology of enamel formation focusing on pathogenic variants underlying AI. We provide a computational model encapsulating new advances in calcium regulation during enamel formation. We also describe the psychological and financial burden of AI, its impact in systemic health, and discuss recent developments in diagnostic panels to detect AI.

1. Introduction:

A common misconception is that the positive effect of maintaining adequate oral health is reflected only on your tooth enamel and a bright smile. As the portal to the rest of the body, healthy teeth and a healthy mouth influences overall health [1]. Inadequate oral care leads to inflammation and infection driven by the action of pathogenic oral bacteria impacting systemic health including the development of cardiovascular disease, respiratory infections and diabetes mellitus, among others [2]. In many ways, dental enamel formation represents a stereotypical model of biomineralization, but it is in many ways unique [3]. Enamel is by far the hardest biological material ever made by cells, harder than dentine, bone and cartilage [4]. Its durability and strength involve a high mineral content and intricate microstructural patterns of interwoven prisms containing thousands of apatitic crystals. Dental enamel is a point of contact during mastication and hence plays a role in digestion, but also in speech, and protects us from various chemical and thermals insults from the food particles and liquids we ingest [5]. Human enamel rarely endures major mechanical failure despite facing challenging mechanical constrains and constant loading during mastication [6]. Healthy teeth and a winning smile inject confidence and have a positive impact on quality of life. Conversely, poor dental care can lead to the progressive destruction of the dental enamel, pain, systemic disease and can have an important psychological burden, especially in the younger population [7]. Improving dental care can help resolve many of these detrimental effects (see clinician's corner). However, pathogenic variants can cause nearly irreparable destruction of the enamel. Such mutations can occur in specific genes abundantly expressed in the enamel forming cells known as ameloblasts, or in other genes that lead to systemic disease. Enamel disease presenting as an inherited abnormal developmental enamel phenotype is known as *amelogenesis imperfecta* (AI) [8,9]. Here, we first provide an overview of how enamel is formed, the pathogenic variants underlying AI and the relevance of calcium in the mineralization process. We address how these variants in key genes involved in enamel formation and those involved in calcium transport affect tooth enamel and the burden of AI. Finally, we provide an update on recently developed diagnostic panels to detect AI.

2. The formation and mineralization of enamel:

Dental enamel is formed and mineralized by ameloblasts, specialized epithelial cells that synthesize and secrete proteins that include those specific to enamel [4,5]. Within the cellular environment of the enamel organ, ameloblasts secrete these proteins forming an extracellular matrix (ECM) in which thin hydroxapatite enamel crystals ($Ca_{10}(PO_4)_6(OH)_2$) form and subsequently undergo expansion [3]. Because

of these two-steps, enamel formation is commonly described as a two-stage process [3,4]. In the first or secretory stage, the secreted ECM forms the volume of the enamel containing thin enamel crystals. This is then followed by the maturation stage, or mineralization stage, in which the matrix is degraded and removed, and ion transport dramatically increases enabling the expansion and increased thickness of the crystals [4]. The ECM and the expansion of the crystals occurs in a controlled depositional environment apical to the ameloblast layer and hence, ameloblasts can regulate this process [3]. Once the maturation stage is completed, most ameloblasts regress or undergo apoptosis, some become incorporated as attachment cells to the gingiva, and any remaining are simply lost during tooth eruption. Ultimately, there are no ameloblasts once the tooth erupts and hence, devoid of cells, enamel cannot undergo self-repair, one of the key differences with bone tissue that can heal and repair through life to various extents [3].

The majority of the extracellular matrix (ECM) secreted by the ameloblasts is composed of the amelogenin protein (90%) (coded by the *AMEL* gene), with small amounts of ameloblastin (*AMBN*) and enamelin (*ENAM*) [10]. Amelogenin is cleaved by two proteases, matrix metalloprotease 20 (*MMP20*), and kallikrein 4 (*KLK4*). AI is commonly caused by pathogenic variants in the genes coding for the ECM components considered critical for normal enamel formation [8]. Within the ECM, thin apatite crystals form in a controlled manner, guided by the constituents of the ECM providing directionality in the growth direction and underlying the microstructural properties of enamel [8]. The maturation stage sees a marked increase in ion transport functions concomitant with stark morphological changes in the appearance of the ameloblasts, which at this point, develop a ruffled morphology with many intricate infoldings of the apical cell membrane that is necessary for proper mineral transport [4]. These ions are extruded from the ameloblasts and likely diffuse and precipitate along the already present thin crystals enabling crystal expansion. Calcium (Ca^{2+}) is the main ion present in the crystal structure of mineralized enamel accounting for ~36% of its mineral content [11].

3. Genetics of enamel disease:

Enamel traits can be inherited as autosomal dominant, autosomal recessive or X linked Mendelian conditions [12]. Different genes and unique pathogenic variants can result in an isolated enamel phenotype, or they can be associated with broader co-segregating phenotypes including syndromes [3]. A nomenclature for these conditions was developed by Carl Witkop Jr in the late 1950s who termed those

conditions affecting only enamel as AI, and all other enamel defects being classified by their syndrome or disease name followed by the presenting enamel phenotype (e.g. hypoplasia) [13,14].

The first reported human genetic mutation associated with an enamel phenotype was in the *AMELX* gene and that codes for the most abundant extracellular matrix protein secreted by ameloblasts was described in 1991 [15]. Since then, numerous additional enamel ECM protein coding genes have been identified with mutations that result in an enamel phenotype (e.g. *ENAM*, *AMBM*, *MMP20*, *KLK4*) [16]. Other genes coding for a variety of proteins with associated enamel phenotypes include, but are not limited to, ion transport regulators, transcription factors, proteinases, cytoskeletal components, cell junction proteins, organelle functions, integrins, and pH regulation elements to name a few [10]. The enamel phenotypes resulting from pathological variants in over 100 human genes are highly variable [8].

The phenotypes range from a slight thinning or pitting of the enamel to mild color changes to severe hypomineralized enamel that crumbles from the dental crown as it emerges into the oral cavity [8]. The most common enamel phenotype listed in OMIM is a deficiency of amount of enamel formed, or hypoplasia [16]. This can manifest as a generalized thin enamel over the entire crown or can be a localized phenomenon characterized by enamel pits or grooves and furrows with surrounding areas of enamel that are of normal thickness. Hypomineralized enamel is another common phenotype and manifests as a discoloration in the enamel that can vary from an opaque white area to a generalized dark yellow-brown discoloration [3,16]. This discoloration results from the altered composition (decreased mineral content, increased protein and water content) and a change in the normal prismatic and crystallite structure of the enamel [17-19]. The mineral content can be so dramatically reduced that the enamel cannot withstand the functional rigors of the oral cavity and is readily lost from the dental crown. These teeth are typically associated with severe dental sensitivity because fully mineralized enamel serves as an excellent insulator that protects the dental pulp from noxious stimuli including irritants and temperature fluctuations [17,18].

In addition to the known genes involving pathologies of enamel, studies show that microRNAs play a role in enamel formation and may contribute to hereditary defects of human enamel [20-22]. MicroRNAs may also play a role in conditions traditionally thought of as environmentally mediated such as dental fluorosis [23]. More recent studies suggest that genetics and the expression of microRNAs could play a role in conditions such as fluorosis making them complex conditions involving both environmental stressors and

human genetics [23]. MicroRNAs could provide a unique approach for therapeutics in the treatment of dental conditions including those involving enamel.

4. Modeling calcium in enamel epithelia

As mentioned above, Ca^{2+} is the main ionic species in enamel, and hence, much work has been recently done to address how ameloblasts control Ca^{2+} transport [11,24]. Ameloblasts transport a substantial amount of Ca^{2+} via an active system that involves Ca^{2+} entry, diffusion, and extrusion at the apical pole [24,25]. Besides, Ca^{2+} must be kept at non-toxic levels and be used as a signaling ion [26]. As is the case for most epithelial cells, Ca^{2+} channels, pumps and exchangers, both in the cell plasma membrane as well as the membranes of intracellular organelles, regulate any surge in cytosolic Ca^{2+} to maintain resting levels around 100 nM [27]. Despite the challenges of working with ameloblast cultures including poor cell viability, recent data has emerged describing functional parameters of individual components of the ameloblasts responsible for the regulation of Ca^{2+} dynamics in ameloblasts [28-32]. To understand how the dynamic Ca^{2+} signaling toolkit operates in ameloblasts and to discern changes in function across stages, it is important to integrate these individual components into a mathematical model to help understand how these processes are connected and how disruptions may lead to enamel disease. Such models will allow us to compare the ameloblasts to other epithelial systems such as salivary glands and pancreas. Moreover, the model can be used to predict the impact of AI-related genes on Ca^{2+} homeostasis, and to design experimental tests to address the use of potential therapeutic targets.

To explore how fluxes are regulated in ameloblasts, we built a computational model [33-35] based on previous observations on the machinery used by ameloblasts to modulate Ca^{2+} transport [11,28,29,31,32,36-38]. Intracellular Ca^{2+} dynamics involve Ca^{2+} exchanges with the endoplasmic reticulum (ER) via the inositol triphosphate receptor (IP_3R) and the sarco-endoplasmic reticulum pump (SERCA) and with the extracellular medium (ORAI, plasma membrane Ca^{2+} ATP-ases (PMCA) and the Na^+/Ca^{2+} exchangers NCX and NCKX) [26] (Figure 1). While PMCA and exchangers mainly extrude Ca^{2+}, ORAI channels mediate Ca^{2+} entry when the Ca^{2+} concentration in the ER decreases, via a sophisticated mechanism known as "Store Operated Ca^{2+} entry (SOCE)". A key element of the functional polarity of the ameloblasts is that the exchangers are mostly expressed at the apical pole, the cell region in close proximity to the forming enamel crystals [4,5]. The model allows to investigate the changes in expression levels of the Ca^{2+} channels, exchangers and pumps and of the cell size, which both characterize

the passage of ameloblasts from the secretory to the maturation stage (see Supplementary Material). In the secretory stage, IP_3Rs, ORAI channels and SERCA's are present in relatively small numbers, while the plasma membrane Ca^{2+}-ATPases (PMCA) are highly expressed [24,39]. Based on this observation, the model predicts that in the absence of IP_3-generating agonist, $[Ca^{2+}]_c$ remains low (0.13 μM) and spatially nearly uniform. Upon stimulation, the Ca^{2+} transient has a relatively small amplitude and decreases slowly (Fig. 1B, dashed lines).

In the maturation stage, Ca^{2+} transporters and exchangers are upregulated, except for PMCA's that are less expressed [24,31,32]. Consequently, the model predicts that basal $[Ca^{2+}]_c$ is slightly more elevated than in the secretory stage and that a Ca^{2+} gradient is established. Such spatial heterogeneity maintains an active transport from the basal to the apical pole. Simulations also predict that in the maturation stage, ameloblasts are very sensitive to a stimulation by an IP_3-generating agonist (Fig. 1B, plain lines). Following Ca^{2+} release from the ER, Ca^{2+} entry via SOCE and diffusion, the net Ca^{2+} efflux at the apical pole is much increased, while in the secretory stage, ameloblasts are importing Ca^{2+} from the extracellular medium in similar conditions (compare plain and dashed lines in Fig. 1C). Although part or this effect is due to the change in the numbers of IP_3Rs and ORAI channels, the smaller size of the ameloblasts in the maturation stage also facilitates Ca^{2+} transport. Taking profit of the flexibility of computational modeling, we performed simulations of an ameloblast with expression levels corresponding to a cell in the maturation stage, but with a cell length corresponding to the secretion stage. Net Ca^{2+} effluxes are reduced by nearly 40% with respect to a less elongated cell (Fig. 1D), although $[Ca^{2+}]_c$ increases are larger (Fig. 1E). Indeed, in this hypothetical system, the larger distance favors Ca^{2+} extrusion of Ca^{2+} by PMCA's, to the detriment of its diffusion to the exchangers in the apical part of the cell. The impact of each component of the Ca^{2+} toolkit can be evaluated separately with the model. As expected by the fact that extrusion is mainly mediated by the exchangers, the model predicts a 4-fold reduction in Ca^{2+} extrusion in cells that do not express NCX and NCKX. However, the model predicts that the most significant reduction (7-fold) of Ca^{2+} extrusion should be observed in cells that do not express ORAI. In such conditions indeed, the limitation in Ca^{2+} entry via SOCE prevents Ca^{2+} transport across the cell. These simulations results agree with the observation that enamel of *Stim1/2*-deficient mice, which cannot sustain SOCE, is severely hypomineralized [29]. More generally, the model can be used to evaluate the consequences at the level of Ca^{2+} homeostasis of mutations of genes involved in Ca^{2+} regulation during amelogenesis, as discussed in the next section.

Many cells utilize a similar composition of the Ca^{2+} signaling toolkits but differ in key properties that could be unique to the required function of the tissue. The modeling of Ca^{2+} dynamics in ameloblasts can be compared with other epithelial cell systems such as pancreatic and parotid acinar cells [40]. Unlike ameloblasts, only IP_3Rs are heterogeneously distributed in the acinar cells. Moreover, as these receptors are type 1 and 2, model simulations display Ca^{2+} oscillations, in line with experimental observations. Another key difference is that these acinar models do not include SOCE, as Ca^{2+} oscillations can occur in the absence of extracellular Ca^{2+}. Whereas for ameloblasts, the model highlights the essential role of the spatial distributions of channels, pumps and exchangers in the ability of these cells to transport Ca^{2+} across the cell, for acinar cells, the models indicate that the phase shift between Ca^{2+} peaks at apical and basolateral membranes plays a key role in the secretion of proteins, enzymes and water. The models even show that, under most conditions, Ca^{2+} diffusion between the two poles of the acinar cells is very limited. In ameloblasts, our current understanding of Ca^{2+} signaling beyond its involvement in mineralization is limited, and hence, whether Ca^{2+} is required for protein secretion is unclear.

5. Calcium-Associated Genes with Enamel Phenotypes

The enamel phenotypes resulting from mutations genes involved in Ca^{2+} regulation during amelogenesis vary from mild to severe [16,41]. Affected enamel is often hypomineralized but can be deficient in amount (hypoplastic), or have both features present (TABLE 1) [42]. Given the importance of Ca^{2+} regulation in normal cellular function [26], mutations in these genes can cause phenotypes that extend beyond the enamel with diverse presentations depending on specific gene functions and cellular/tissue expression. For example, *STIM1* (OMIM# 612783) and *ORAI1* (OMIM# 612782) mutations are associated with immunodeficiency [43]. Timothy syndrome (OMIM# 601005) caused by mutations in the *CACNA1C* gene is associated with digit webbing, immune deficiency, and multiorgan dysfunction including heart and neurological/ cognitive abnormalities [44]. In other conditions, the clinical phenotype appears limited to the enamel and is thus classified in OMIM as a form of amelogenesis imperfecta including types IIA5, IIA6 and 1H as listed in TABLE 1 that includes some of the pathogenic variants associated with Ca^{2+} homeostasis [42].

These conditions are rare, so detailed phenotyping of the enamel and dental manifestations is limited. The hypoplastic enamel phenotype can be generalized thin, or it can be a thin, rough, and/or pitted phenotype as seen with Timothy syndrome and amelogenesis imperfecta type 1H. Mutations in genes directly

associated with Ca^{2+} transport in enamel include *SLC24A4* and *STIM1* [29,32,45-47] which are characterized by discolored, hypomineralized enamel (Figure 2). These defects are currently classified as hypomaturation defects because these genes are expressed specifically in the maturation stage of enamel development [47]. Mutations in other genes associated with Ca^{2+} homeostasis but less known in enamel include *ITGB6* (integrin subunit beta 6) which can have both enamel hypoplasia and hypomineralization with the enamel frequently chipping away from the dental crown as it fractures from the forces of use [48], mutations in the proton sensing *GPR68* (G protein-coupled receptor 68) [49]. *CNNM4* (ancient conserved domain protein 4 gene) is a magnesium transporter that affects Ca^{2+} levels in some cells [50] and mutations cause Jalili syndrome with dysplastic/discolored enamel *[51]* (Figure 2).

6. The burden of enamel disease:

The face is equipped with muscles whose function is almost entirely dedicated to engage in facial expressions and the display of emotions [52], including the simple act of smiling [53]. Aesthetics are integral parts of an individual's personality and poor dental health, and esthetics, create an important psychological burden that affects self-esteem, limiting social interactions including social avoidance, and generally impacting quality of life, especially among the younger population [53,54]. The affliction of such burden is captured in the following statement by a patient with AI: "*I wouldn't have children knowing I would give them AI. I know the issues it brings, and I couldn't stand to see my child go through both emotional and physical pain as I did.*" (A.M. pers comm). Beyond this important psychosocial burden, global estimates of the annual financial costs of oral diseases in 2015 by World Health Organization are more than $500 billion, of which nearly half was associated with dental caries [55], the most common non-communicable disease affecting about 2 billion people worldwide [56]. AI requires a high burden of dental care [57], and although data on the costs of AI are difficult to establish, a recent study reported that the costs of managing dental caries in individuals ranging from 12 and 65 years old in Brazil, France, Germany, Indonesia and Italy ranged from over $10 billion in Italy to over $36 billion in Brazil [58]. The largest per-person costs for all procedures associated with dental work was in the UK at nearly $23,000 [58]. Other reports indicate that in the UK alone, (period of 2021-2022) £81 million were spent in tooth extractions associated with caries in children less that 19 years of age [58]. In the US, The National Institute of Dental and Craniofacial Research reported that over a lifetime, dental care averages ~$51,000 for a single coverage [59]. Beyond the costs of dental care, a 2018 study estimated that the loss of school hours in young individuals exceeds $320 million [60].

Pathological variants in Ca^{2+}-regulating genes that alter tooth formation are associated with significant morbidity and, in cases such as Timothy Syndrome, increased mortality [61]. Reports on these conditions can include an increased prevalence of dental caries and in some cases tooth loss [61]. The affected individuals often have increased dental sensitivity, and diminished oral function and ability to masticate. Tooth and enamel loss, and malocclusion are more prevalent in people with hereditary enamel defects. Correcting the dental conditions varies in approach being predicated on the clinical phenotype. It often requires multiple stages of dental restorations that can begin with the primary dentition and continue through young adulthood as the primary dentition is replaced by permanent teeth. Orthodontics and even orthognathic surgery are not uncommonly needed to correct malocclusions and produce a functional articulation of the teeth and dental arches to improve masticatory function. Once all permanent teeth have erupted there is then the need to maintain oral health throughout adulthood. The lifetime cost of oral care will depend on the phenotype and care needed and desired but can be quite substantial [62].

7. Genomic testing of enamel disease.

The translation of genomic knowledge to patient care reflects recognition by policy makers, clinicians and healthcare users of the added value brought by precision diagnosis. There is a patient appetite for application of this new knowledge to their care. Recent years has seen a shift from clinical genomics being restricted to specialist services (e.g. Clinical Genetics) to an expectation that genetic testing is offered more widely by doctors, dentists and other healthcare professionals. For developmental enamel disease, that in some instances includes other co-segregating disruption to tissue/organ formation or function, application remains focused on AI genetic testing. This is available via two broad routes: commercial companies and healthcare systems. Commercial companies have been quick to bring testing to the global market for several genes where variants may cause AI. Typically, these are offered as individual gene or small AI panel tests to parameters and standards set by individual providers. Each AI panel may not include all relevant gene tests offered by that provider with potentially pertinent genes included in other panels without an explicit link to tooth development. Test requests mostly require involvement of a clinician such as a doctor. Government sponsored approaches to genomic medicine are developing in several countries with a few having implemented testing for personalized and precision medicine that is continuing to evolve [63]. The National Healthcare Service (NHS) Genome UK policy represents an example of a healthcare system approach to genomic healthcare and research. The R340 Amelogenesis

Imperfecta panel is one of 6 Musculoskeletal panels that continue to evolve. At the time of writing, R340 tests 41 genes including several with important roles in Ca^{2+} homeostasis and mineralization. Requests can be made only by selected clinicians such as specialists in Pediatric Dentistry. Testing is free at the point of delivery reflecting a nationally commissioned service funded by UK tax receipts. All NHS panel tests, which in total cover over 4,000 genes, are undertaken, reported and quality assured to the same standards within a single governance framework. An aligned research approach increases opportunities for ambitious, cross-disciplinary discoveries. In the US, genetic testing for AI is also available through several laboratories including Prevention Genetics, offering a panel testing for 34 genes, Fulgent Genetics (42 genes), Blueprint Genetics (16 genes), and others.

The increasing uptake in clinical care of genetic testing brings new understanding and opportunities to advance healthcare planning and delivery, including for those who do not have access to testing. However, careful consideration of the potential impacts on all AI genetic testing stakeholders is needed: individual being tested and their wider family, clinicians requesting testing, and healthcare systems (including insurance). The complexities are well understood yet can be difficult to implement into routine clinical care, especially where genetic testing is new to a particular group of clinicians. Technical approaches to genetic testing and result interpretation for diagnostic clinical care continue to develop. For example, the UK NHS is increasingly deploying whole genome sequencing (WGS) as part of routine care. The advancing sophistication needs to be matched with requesting clinician currency in contemporaneous use. This includes clinicians being prepared to manage the expectations and experiences of those being tested within systems that will increasingly require clinical utility and value for money. The integration of AI genetic testing into mainstream clinical care is in its infancy. Discoveries from genetic testing across multiple clinical disciplines can be expected to advance the understanding of enamel formation and how this fails, including via disruption of calcium homeostasis.

7. Conclusions and future research.

Genetic mutations affecting enamel formation leads to a vast economic and psychosocial burden. Because of this, there has been an interest by federal agencies and pharma companies to develop genetic testing for AI. The UK and France lead the way in AI testing, but it is also available in the US. The development of these panels is now possible because of a better understanding of the biology of ameloblasts and the identification of genes and molecular pathways regulating enamel formation, which has expanded

significantly in the last decade. Some mutations in the enamel genes result in phenotypic changes only in enamel without co-occurring defects in other cells or organs in the body. However, several gene mutations generally ascribed to diseases occurring in specific organs, may also affect ameloblast function and hence enamel formation (see "Outstanding questions"). Some of these mutations are associated with disruptions in Ca^{2+} transport.

In recent times, we have seen a continuously growing list of genetic mutations affecting enamel formation. Therefore, since the time that Witkop elaborated a classification system for enamel defects, our understanding of the underlying genetic mutations and molecular pathways, the corresponding enamel phenotypes has provided a picture which suggests that the current classification system needs revision. This will help clinicians to better assist in patient care and will aid in continuing the development of newer and more standardized panels for genetic testing of AI, helping reduce the economic and social burden of enamel disease (see "Outstanding questions"). This would be a remarkable achievement but requires sustained plans for continuing dental enamel research.

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Conflict of interest

The authors declare no conflict of interest

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Figure legends

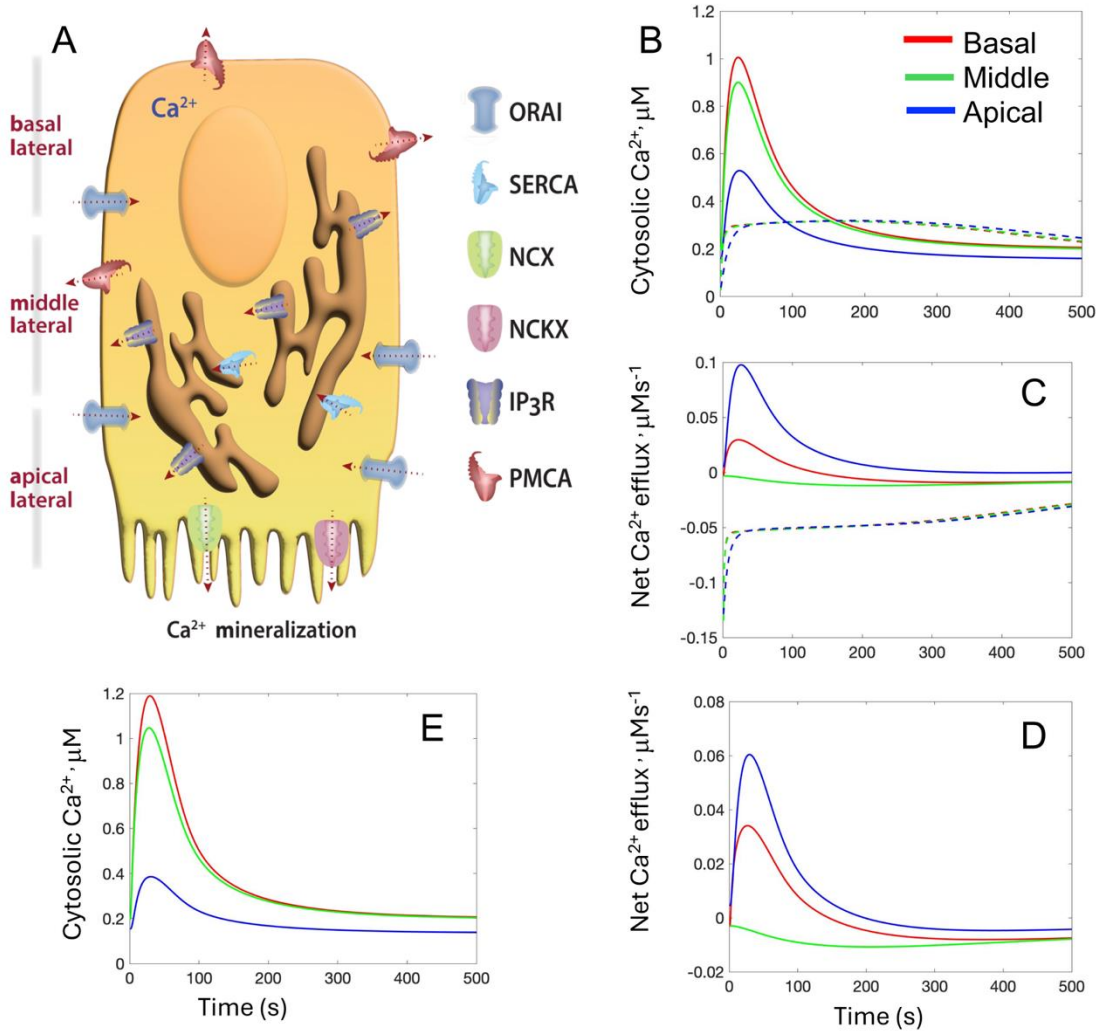

Figure 1: **Computational modeling of Ca^{2+} dynamics in ameloblasts. (A)** Schematic representation of the Ca^{2+} fluxes considered in the model. The densities of IP_3R, ORAI channels, PMCA's and SERCA's are larger in the basal and central parts of the cell, while NCX and NCKX are more expressed in the apical pole. See Supplementary Material for a full description of the model, equations and values of parameters. **(B)** Time evolution of $[Ca^{2+}]_c$ in response to a transient activation of PLC. Plain and dashed lines refer to ameloblasts in the maturation and secretory stages, respectively. The different colors refer to the $[Ca^{2+}]_c$ in the different parts of the cell (basal pole, central region, apical pole). **(C)** Time evolution of the net outward fluxes in the same conditions as in panel B. These fluxes are computed as the sum of the fluxes through PMCA's and exchangers minus the flux through ORAI, for each region of the cell. A positive value indicates that more Ca^{2+} is going out than coming in the cell. **(D)** Net outward fluxes in the hypothetical situation of an ameloblast characterized by channels and transporters densities corresponding to the maturation stage but having the spatial extent of an ameloblast in the secretion stage. Cell lengths are 70 mm and 40 mm, in the secretory and maturation stages, respectively. **(E)** Evolution of $[Ca^{2+}]_c$ in the same conditions as in panel D.

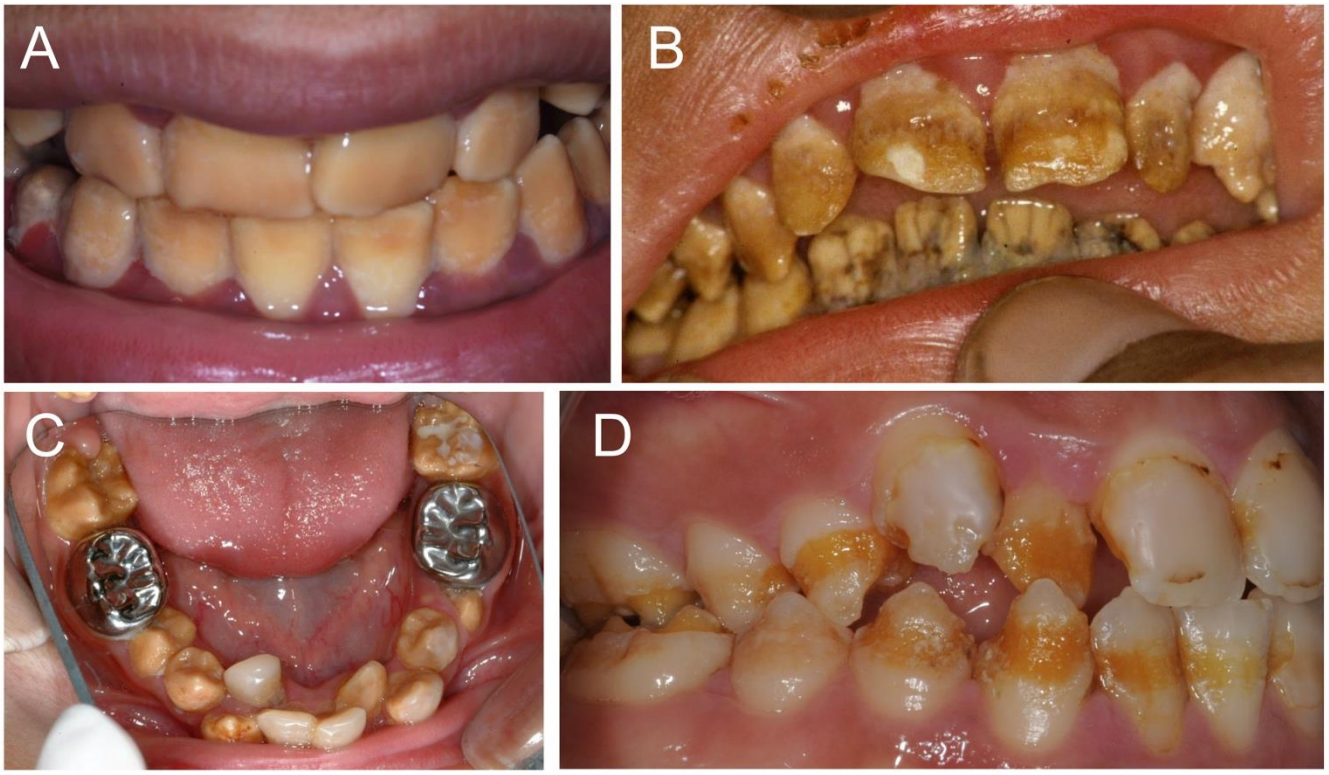

Figure 2. Examples of hereditary enamel disorders due to disruption of ion transport. (**A-D**). In the secondary (adult) dentition typical shared features are near-normal enamel volume that is not fully mineralised. On tooth eruption the enamel is opaque with creamy colouration. Post-eruption changes affect the colour with darkening through shades of brown and compromised enamel integrity that includes cracks and loss of enamel down to the dentine influenced by a range of factors. Underlying pathological variants: (**A)** *SLC24A4*, (**B)** *CNNM4*, (**C)** *STIM1*, (**D)** *ITGB6*. Note. Dental restorations, including ones that are tooth coloured, need to be considered in assessment of the dental phenotype. Recognition of a possible abnormal dental phenotype should prompt assessment by a dentist.

TABLE 1. Pathological variants in Ca^{2+} associated genes affecting enamel

Condition & OMIM #	Gene	**Gene Function**	**Enamel Phenotype**
Amelogenesis Imperfecta Type 1H OMIM# 616221	*ITGB6*	RGD- and calcium-dependent vitronectin receptor	Hypoplastic and Hypomineralized
Amelogenesis Imperfecta Type IIA5 OMIM# 615887	*SLC24A4*	potassium-dependent sodium/calcium exchanger	Hypomineralized (listed as hypomaturation)
Amelogenesis Imperfecta Type IIA6 OMIM# 601404	*GPR68*	mobilize calcium from intracellular stores	Hypomineralized (listed as hypomaturation)
Enamel Renal syndrome OMIM# 611062	*FAM20A*	Calcium metabolism	Hypoplastic and Hypomineralized, can have abnormal tooth eruption
Immunodeficiency, ectodermal dysplasia OMIM# 612782	*ORAI1*	plasma membrane protein forming a Ca^{2+} channel essential for store-operated calcium entry	Hypomineralization
Immunodeficiency, ectodermal dysplasia OMIM# 6125887	*STIM1*	Calcium sensor/transport/activates ORAI channel	Hypomineralization
Timothy Syndrome OMIM # 601005	*CACNA1C*	Contribute to voltage-gated calcium channel	Hypoplastic/hypomineralized Taurodont teeth
Jalili syndrome OMIM # 607805	*CNNM4*	Magnesium transporter	Ddysplastic/discolored enamel