

Molar incisor hypomineralization - a multifactorial condition?

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ABBREVIATIONS

AI	Amelogenesis imperfecta
ADHCAI	Autosomal dominant hypocalcified amelogenesis imperfecta
AMBN	Ameloblastin, protein coding gene
AMELX	Amelogenin, x-linked, protein coding gene
BMP	Bone Morphogenetic Protein
DDE	Developmental defects of enamel
DEJ	Dentino-enamel junction
DLX3	Distal-less-homeobox3
EDCs	Endocrine disrupting chemicals
ENAM	Enamelin, protein coding gene
FAM83H	Family with sequence similarity 83 member H
FGFR1	Fibroblast Growth Factor Receptor1
FPM	First permanent molar
KLK4	Kallikrein related peptidase 4, protein coding gene
MMP20	Matrix metalloproteinase 20, protein coding gene
MIH	Molar Incisor Hypomineralization
NNL	Neonatal line
PD	Predentine
PEB	Posteruptive-breakdown
SEM	Scanning electron microscopy
TUFT	Tuftelin, protein coding gene
TFIP11	Tuftelin-interacting protein 11, protein coding gene

INTRODUCTION

While the prevalence of caries in children in European countries has decreased over the past several years (1), interest in developmental enamel defects has been growing, in particular in isolated defects in enamel of first permanent molars (FPM) and incisors. At the end of the 1970s an acute form of idiopathic hypomineralization affecting FPMs and permanent incisors was first mentioned in the literature (2). Other studies also reported similar defects; these were described as demarcated opaque regions with changed colour most often localized on the occlusal surfaces. Many different terms have been used to refer to these developmental defects of the enamel in the FPMs and incisors: “idiopathic enamel hypomineralization”(2, 3), “hypomineralized first permanent molars” (4), “demineralization of the first permanent molars”(5), “non-fluoride enamel hypomineralization in the first permanent molars”(6), or “cheese molars”(7, 8).

In 2001 Weerheijm and co-workers defined the phenomenon as a hypomineralization of systemic origin of one to four permanent first molars frequently associated with affected incisors and suggested the name Molar Incisor Hypomineralization (MIH) (9). Most of the literature so far published on MIH has been case-control or retrospective cohort studies focusing on the prevalence of MIH. A review from 2010 reported that the prevalence of MIH varies from 2.4% to 40.2% (10). However, the author stated that the cross comparison of the results of the various studies were difficult because of the use of different indices and criteria, examination variability, methods of recording and different age groups. Only a few studies have evaluated possible aetiological factors. Although pre-, perinatal or early life illnesses or events have been implicated, likely systemic causes have not been identified. However, it has been suggested that MIH may have a multifactorial aetiology, with different factors acting additionally or even synergistically, and with a genetic predisposition associated with one or more systemic insults occurring at a susceptible stage in the development of specific teeth (11-13).

This Master Thesis is divided into 4 parts; the first part describes enamel formation and structure, where the different genes responsible for this formation are presented. Various developmental enamel disturbances are also briefly mentioned. Parts II and III provides basic information concerning MIH, including clinical and histological findings. It also provides an

overview of several studies focusing on the aetiological aspects of MIH. Finally, the last part consists of an overall summary of the different studies presented in this assignment.

PART I

ENAMEL FORMATION

Human teeth are composed of three different mineralized tissues: cementum, dentine, and enamel. Dentine and enamel formation take place simultaneously, and both processes start along a line that will become the dentino-enamel junction (DEJ) (14). The process of enamel formation, also called the amelogenesis, occurs in stages, beginning at the cusp tips or incisal edges and progressing along the sides of the crown toward the cervical margin (Fig. 1). The amelogenesis include the pre-secretory, secretory, transition and maturation stages. The enamel-forming-cells, the ameloblasts, change both morphology and function as they progress through each stage (15).

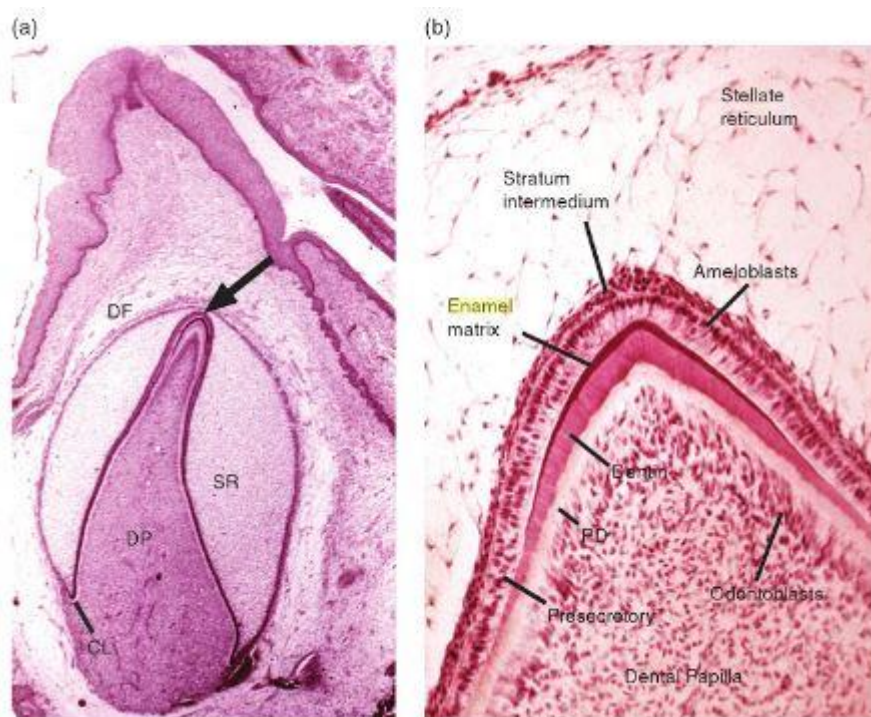


Figure 1. (a) Low magnification of a developing incisor in the early crown stage, with deposition of enamel and dentine matrices at the cusp tip (arrow). Cervical loop (CL), dental follicle (DF), dental papilla (DP), stellate reticulum (SR). (b) Higher magnification of a similar developing cusp tip. Secretory ameloblasts have differentiated at the cusp tip and have deposited a thin layer of enamel matrix on the outer surface of the dentine. Along the sides of the cusps, closer to the cervical loop, the ameloblasts are in the pre-secretory stage of differentiation, and at the lower left inner enamel epithelial cells are seen. Predentine (PD).

(The figures are copied from Hand AR, Frank ME. Fundamentals of oral histology and physiology: Wiley-Blackwell; 2014)

Pre-secretory stage

The pre-secretory stage involves the differentiation of ameloblasts from the inner enamel epithelial cells and the resorption of the basal lamina (Fig. 2). An elongation and polarization of the cells occurs due to a relocation of the nucleus to the apical end of the cell, migration of the Golgi complex to the supra-nuclear cytoplasm, and an increase in the amount of rough endoplasmic reticulum. These cells are then called pre-secretory ameloblasts and, have not yet begun to secrete enamel matrix.

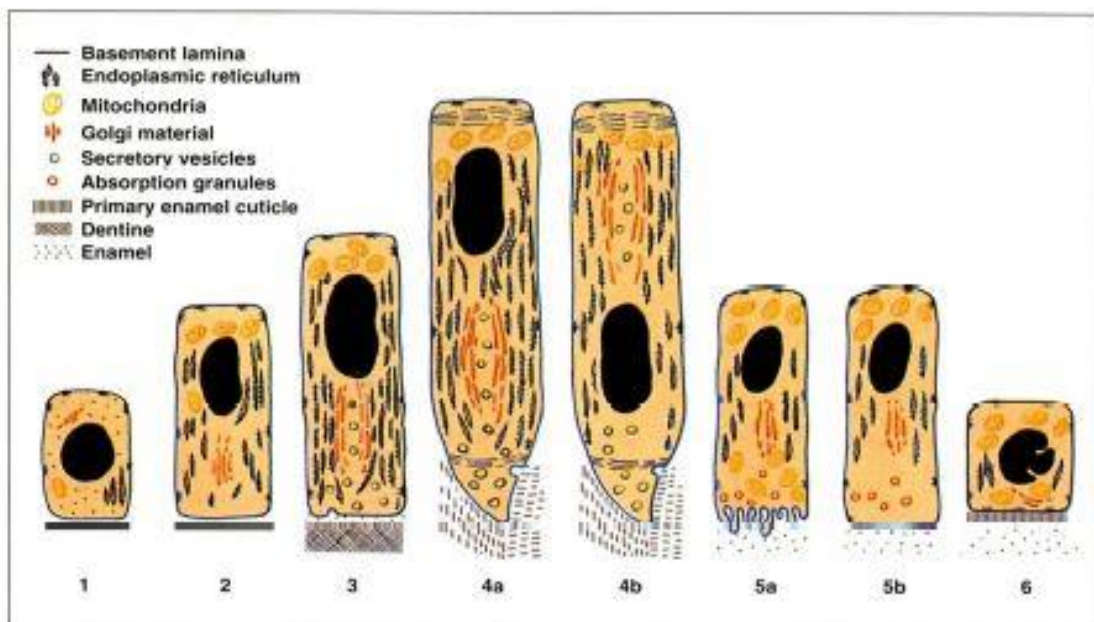


Figure 2. The life cycle of an ameloblast. The cells of inner enamel epithelium (1) start to differentiate at the future cusp tip. The differentiating cell becomes columnar (2) and the nucleus moves to the part of the cell furthest from the dentine. The cell starts to secrete the initial enamel matrix (3), and as it retreats the Tomes process develops (4a). In this phase two appearances of the cell can be distinguished by the position of the nuclei within the cell: high (4a) and low (4b). When the full thickness of enamel has formed, ameloblasts lose the secretory extension, the Tomes process (5a). During the maturation phase there is a regular, repetitive modulation of cell morphology between a ruffled (5a) and a smooth (5b) surface opposed to the enamel. Once the maturation is complete, the cell regresses in height (6). (The

figure is copied from Berkovitz BKB, Moxham BJ. Oral anatomy, histology and embryology. London; Baltimore: Mosby-Wolfe; 1994. p.144)

Another feature of this stage is the presence of a basal membrane that separates the pre-ameloblasts from the dental papilla and odontoblasts. This marks the future enamel-dentine junction. Odontoblasts, the cells forming and maintaining dentine, differentiate as a result of signal by the inner enamel epithelium. When the first layer of dentine has formed, enzymes are excreted from the dental papilla and the basal membrane is broken down. Immediately after degradation of the basal lamina, ameloblasts and odontoblasts are in direct contact and can exchange signals (16). When the odontoblasts start to lay down their dentine a signal is given that stimulates the ameloblasts to start secretion of enamel matrix that later will become mineralized (17). The initial layer of enamel matrix is aprismatic due to the fact that the Tomes` process has yet not been differentiated.

Secretory stage

The detailed structure of the secretory stage ameloblasts reflects their intense synthetic and secretory activity. The pre-ameloblasts transform into secretory stage ameloblasts by both elongating into tall columnar cells and forming Tomes` processes at their apical ends nearest the forming enamel. The Tomes` process is a conical structure that points toward the forming enamel matrix. Enamel matrix proteins are primarily secreted from one side of the Tomes` process (secretory face), and all ameloblasts within a row secrete protein from the same side of their Tomes` processes (18). The ameloblasts start secreting large amounts of enamel matrix proteins as they move away from the dentine surface so that the recently made enamel layer can increase in width (17).

In addition to moving away from the dentine matrix as the enamel matrix thickens, the ameloblasts also move in groups that slide by one another, and this movement culminates in the characteristic decussating enamel prism pattern observed in rodent incisors (19) or the entwined gnarled prism pattern seen in human molars (17). As this occurs, ameloblasts secrete four different proteins into the enamel matrix, where three are presumed structural proteins and one is a proteinase. The structural proteins are amelogenin (encoded by the *AMEL* gene), ameloblastin (encoded by the *AMBN* gene), and enamelin (encoded by the *ENAM* gene), and

the proteinase is matrix metalloproteinase-20 (encoded by the *MMP20* gene). Amelogenin comprises approximately 80–90% of the organic matter within the secretory stage enamel matrix, and ameloblastin and enamelin comprise about 5% and 3–5%, respectively (20). MMP20 is present in trace amounts. Later on, one more protease is secreted into the enamel matrix of developing teeth. The late protease is kallikrein 4 (*KLK4*), which is secreted by transition- and maturation-stage ameloblasts. *KLK4* aggressively degrades the retained organic matrix following the termination of enamel protein secretion. Proteins and an organic matrix form a partially-mineralized enamel in the secretory stage. However, it is not until the end of the maturation stage when the proteins are almost completely removed, that the enamel achieves its final mineralized form.

Transition stage

During the transition stage, before the enamel layer reaches its full thickness, ameloblasts undergo changes in form and function in order to contribute to the maturation process. Ameloblast height is reduced and the number of cells decreases to 50% due to apoptosis. The matrix-producing cell organelles disappear by autophagocytosis. In addition, they retract their Tomes' processes, smooth off the enamel surface with a final coating of aprismatic enamel, transition into shorter and fatter maturation stage cells, and, reapply a new basal lamina, as well as start modulating between ruffle and smooth-ended cells at the enamel surface (21). The immature enamel is now complete, consisting of 65% water, 20% organic material-like proteins and 15% inorganic hydroxyapatite, and is therefore very porous. A maturation process is required to make the hard final mature enamel.

Maturation stage

In the maturation stage of amelogenesis the enamel proteins are degraded and further mineralization occurs. In addition to secreting calcium, phosphate and carbonate ions into the matrix, the ameloblasts remove water and degraded proteins from the matrix. Enamel crystallites increase in width and thickness at the expense of intercrystalline space (22). During this stage the protein removal and mineralization is completed. In a post-maturation stage, the enamel organ finally retires and the tooth erupts into the oral environment.

ENAMEL STRUCTURE

Mature enamel is highly mineralized, consisting of 96% mineral and 4% organic material and water, and its structure is therefore difficult to study. Examination of conventional demineralized sections shows only empty spaces in areas previously occupied by mature enamel, because the mineral has been dissolved and the trace organic material has also been washed away. However, scanning electron microscopy (SEM) technology has offered many advantages to study the structure of enamel as it provides a greater depth of focus and more detailed resolution than light microscopy (23).

Enamel is the only epithelial-derived calcified tissue in mammals and its structure is unique because of its high mineral content. Enamel is made up of highly organized, tightly packed hydroxyapatite crystallites, $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, that compromise 87% of its volume and 95% of its weight (15). Whereas other mineralized tissues consist of about 20% organic material, mature enamel contains less than 1% organic matter (17). Enamel crystals are extremely long relative to their thickness and highly oriented, generally extending from the underlying dentine toward the surface of the tooth. The crystals are organized into bundles, called prisms, and both this organization and mineralization give the dental enamel its outstanding physical properties.

During enamel formation, the ameloblasts move away from the enamel-dentine junction towards the surface of the enamel. Each single ameloblast makes an individual contribution to enamel production. However, the layered building of enamel is a joint venture of a continuous sheet of ameloblasts, the ameloblastema. The incremental lines of enamel, the Retzius lines (striae), represent the movement of the ameloblastema (Fig. 3), while the path pursued by each individual ameloblast is traced out by the prisms (24). The fine, horizontal grooves on the surface of the crown, the perikymata grooves, represent the external manifestations of the Retzius lines (Fig. 4). The number of perikymata grooves on the side aspect of a tooth is identical to the number of Retzius lines that reach the enamel surface (25). When the very sensitive ameloblasts are subjected to a noxious episode of either internal or external origin, a temporary change in the rhythmic enamel matrix formation may occur, causing some striae of Retzius to appear more prominent than normal. An example of accentuated Retzius line that corresponds to the event of birth is known as the "neonatal line" (NNL) (26).

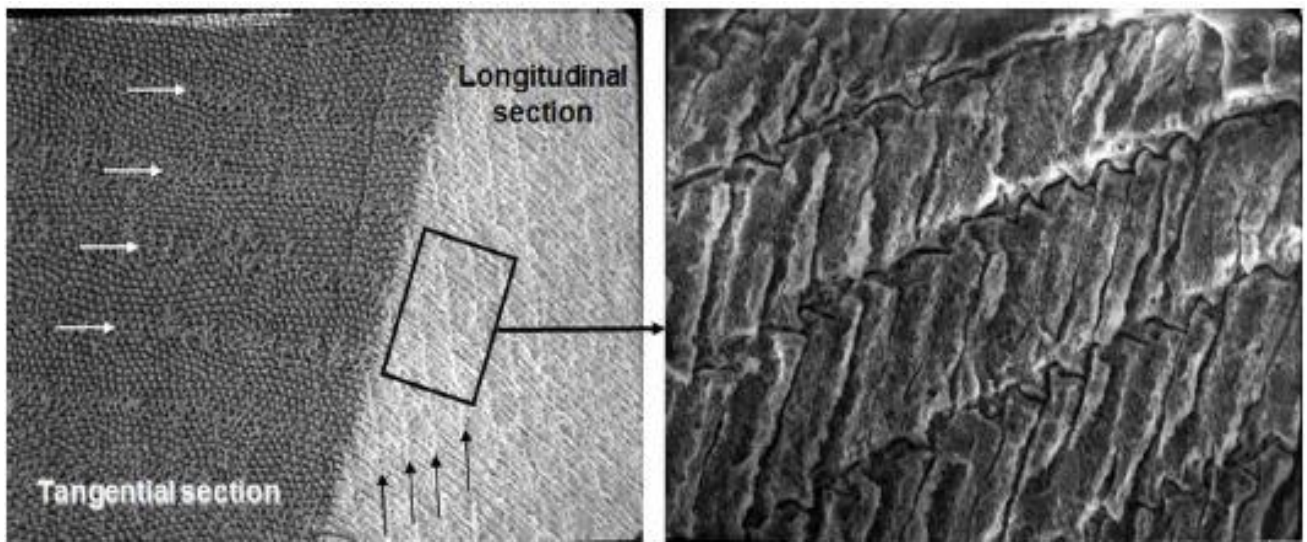


Figure 3. Scanning electron microscope images of human enamel exhibiting Retzius line showing the region where two ground planes meet. Retzius lines in outer enamel (arrows) are continuous across the area where tangential and longitudinal planes meet. (The figures are copied from Risnes S. Growth tracks in dental enamel. *J Hum Evol.* 1998;35(4-5):331-50.)

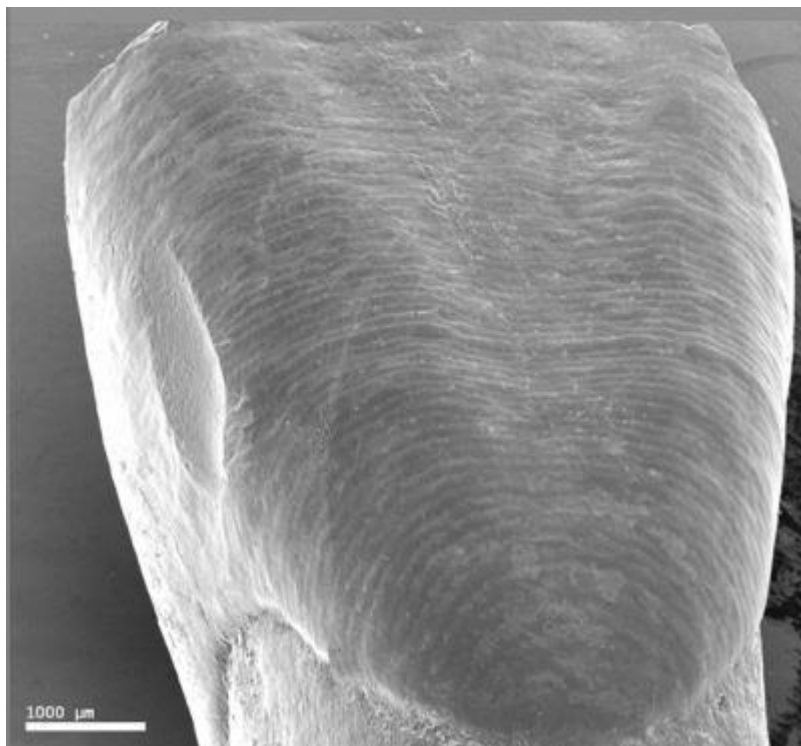


Figure 4. Scanning electron micrograph of perikymata on a *Paranthropus robustus* (early hominin) incisor tooth surface. (The figure is copied from Dean MC, Reid DJ. Perikymata spacing and distribution on hominid anterior teeth. *Am J Phys Anthropol.* 2001;116(3):209-15)

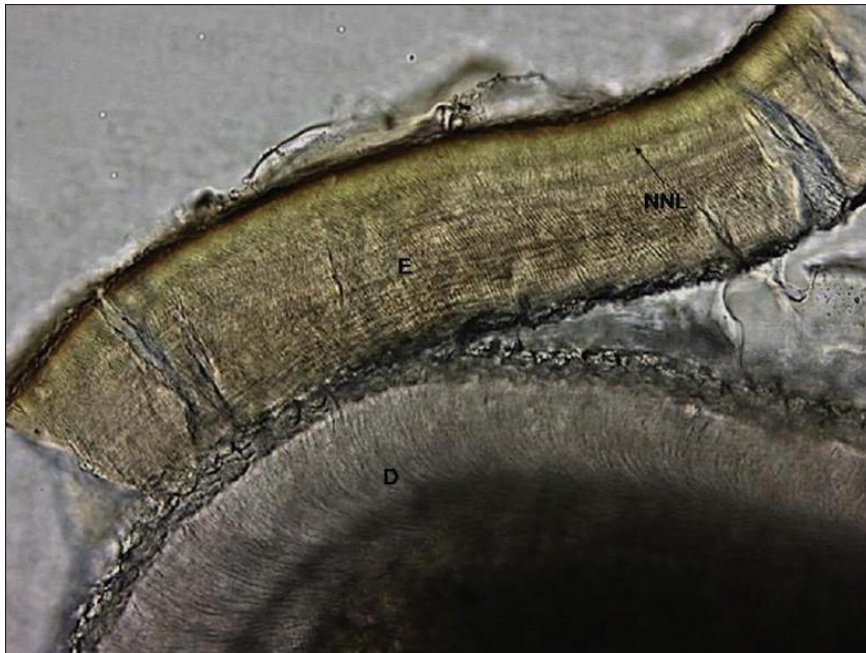


Figure 5. Light microscopic view of ground section of mandibular central incisor tooth germ exhibiting a neonatal line (NNL-Neonatal line; E-Enamel; and D-Dentine). (The figure is copied from Janardhanan M, Umadethan B, Biniraj K, Kumar RV, Rakesh S. Neonatal line as a linear evidence of live birth: Estimation of postnatal survival of a new born from primary tooth germs. *J Forensic Dent Sci.* 2011;3(1):8-13.)

ENAMEL GENES

Genes responsible for enamel formation have been proposed as potentially involved in caries susceptibility (27), but they have also been proposed to play a role in the incidence of hereditary enamel defects such as amelogenesis imperfecta (AI) (28). This part of the thesis focuses primarily on candidate genes involved in enamel formation. As mentioned previously, the major enamel matrix proteins involved in enamel formation are amelogenin, enamelin and ameloblastin, and the genes encoding them are respectively *AMEL* gene, *ENAM* gene and *AMBN* gene. The proteases that are responsible for processing these proteins include *MMP20* and *KLK4*. In addition, the Family with sequence similarity 83 member (FAM83H) gene and tuftelin genes and proteins have been shown to play an important role in enamel formation.

Amelogenin

Amelogenin (encoded by *AMEL* gene) is the major extracellular matrix protein of developing enamel, and represents 90% of the organic content (29). It is thought to be critical for the formation of normal enamel crystallite morphology. Normally, amelogenin is first secreted by ameloblasts, and is subsequently removed by enamel-specific proteinases (30). Amelogenins are believed to function as the principal organizers of enamel deposition, but more recently they have been suggested to be also involved in root formation, periodontium regeneration and to function as growth factors (31). In humans, *AMEL* is encoded by two genes located on the sex chromosomes, *AMELX* and *AMELY*. The *AMELX* gene, which is located on the X chromosome, makes almost all of the body's amelogenin. The copy of the amelogenin gene on the Y chromosome, *AMELY*, makes very little amelogenin and is not needed for enamel formation. (32).

Enamelin

Enamelin (encoded by *ENAM* gene) is the largest enamel matrix protein, and has been detected both in the secretory ameloblasts and in the developing enamel matrix (33). Enamelin functions at the mineralization front where the enamel mineral ribbons initiate and lengthen, along the outer surface of the ameloblast distal membrane where enamel proteins are secreted. In the absence of enamelin, the mineralization front fails and enamel crystals do not form. Instead, mineral deposits slowly, within a layer of accumulated enamel protein and

in the intercellular spaces between secretory ameloblasts, resulting in a crusty material covering the coronal dentine that cannot support mastication and readily abrades away (34). Enamelin is thought to be the main candidate gene responsible for the autosomal-inherited form of AI, while mutations in amelogenin lead to X-linked AI (35).

Ameloblastin

Ameloblastin (encoded by *AMBN* gene) represents about 5% of the enamel matrix content and has been detected both in the secretory ameloblasts and in the entire thickness of the enamel matrix (36). Studies carried out on the interaction between ameloblastin and amelogenin suggest a co-operative function in the scaffolding needed for formation of enamel (37). Mouse recombinant ameloblastin is shown to act as a growth factor increasing cell attachment and proliferation of periodontal ligament cells *in vitro*. Ameloblastin has also been found in pre-odontoblasts, pulpal mesenchymal cells and Hertwig's epithelial root sheath cells, but its function in these tissues as well as in ameloblasts is still not fully understood (38).

Matrix metalloproteinase-20

Matrix metalloproteinase 20 (encoded by *MMP20* gene), also known as Enamelysin, is a member of the "matrix metalloproteinases"-family (MMPs). Enamelysin is the foremost enamel matrix-processing enzyme. It is expressed prior to the onset of dentine mineralization and expression continues throughout the secretory stage of amelogenesis. *In vitro*, enamelysin catalyses all of the amelogenin cleavages that are known to occur during the secretory stage *in vivo*, and it is probably the enzyme responsible for the processing of all enamel proteins (39). There is evidence suggesting that enamelysin activity is critical for proper enamel formation. Un-cleaved and processed enamel proteins often segregate into different compartments within the developing enamel layer, suggesting that they may have different functions (40). MMP 20 is responsible for most of the proteolytic activity of the enamel matrix by starting the hydrolysis of the enamel matrix proteins. During this process a lot of enamel matrix is removed and this gives room for the enamel crystals to grow.

Kallikrein 4

Enamel matrix serine proteinase 1 (EMSP1), now officially designated kallikrein related peptidase 4 (KLK4), is believed to be the predominant degradative enzyme that clears enamel proteins from the matrix during maturation. The *KLK4* gene is initially expressed during the transition stage and continues to be expressed throughout maturation. KLK4 concentrates at the enamel surface when the enamel matrix disappears, and aggressively degrades amelogenin *in vitro* (40).

FAM83H

FAM83H (family with sequence similarity 83 member H), is a gene in humans that encodes an intracellular protein, known as FAM83H, with unknown function but appears to be associated with the Golgi apparatus or trans-Golgi network and is most strongly expressed by pre-ameloblasts (41). The gene is expressed in many tissues; however, all mutations reported to date result only in enamel abnormalities, suggesting that this gene is essential for enamel formation, but is not as critical in other tissues (42, 43). Based on its expression pattern, Lee and co-workers suggested that FAM83H might be involved in the differentiation of pre-ameloblasts into functional ameloblasts and in enamel matrix calcification (41). The FAM83H protein is required for proper dental enamel calcification (42). Recently, Zhang and co-workers showed that FAM83H could influence enamel biomineralization and dentine formation, and they reviewed studies that identified different mutations in the FAM83H gene associated with autosomal dominant hypocalcified amelogenesis imperfecta (ADHCAI) (44).

Tuftelin

Tuftelin (encoded by *TUFT1* gene) is another protein that has been suggested to play an important role during the development and mineralization of enamel, but its precise function is still unclear. Tuftelin is also expressed in several non-mineralizing soft tissues, suggesting that it has a universal function and/or a multifunctional role (45). Tuftelin-interacting protein 11 (TFIP11) was first identified in a yeast two-hybrid screening as a protein interacting with tuftelin. The ubiquitous expression of TFIP11 suggested that it might have other functions in non-dental tissues (46).

DEVELOPMENTAL DISTURBANCES OF ENAMEL

Developmental defects of enamel (DDE) are not uncommon; they can be seen both in the primary and permanent dentitions. DDE remain as a permanent record of a disturbance during amelogenesis. These defects may be inherited as mutations in the genes that code for enamel proteins, or as a feature of generalized familial conditions. The possible aetiological factors for enamel defects in permanent teeth can be broadly divided into two main categories: those with a localized distribution and those with a generalized distribution. Amongst the causative agents of localized defects of enamel are trauma, localized infection and irradiation. Amongst the causative agents of generalized defects of enamel are genetic disorders and systemic disturbances including intoxications (47), perinatal and postnatal problems, malnutrition, infectious diseases and a range of other medical conditions (48) (Fig. 6).

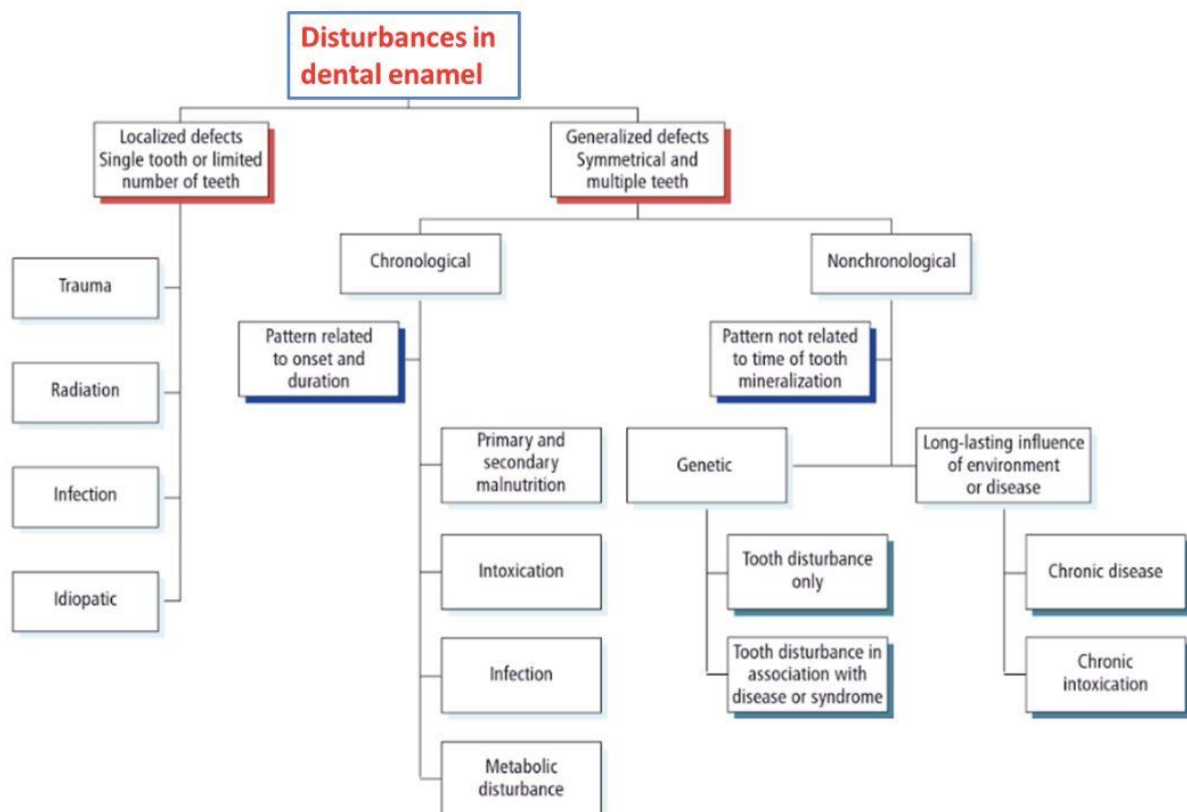


Figure 6. Aetiological factors responsible for the formation of enamel defects. (The figure is copied from Koch G, Poulsen S. *Pediatric Dentistry: A Clinical Approach*. 2nd ed. Copenhagen: Munksgaard: Wiley; 2001)

Wide variations exist in the literature because of the use of various terminologies and the different diagnostic criteria employed to describe the enamel defects in the permanent dentition (49, 50). Nevertheless, the majority of reports have failed to demonstrate any difference in the prevalence of enamel defects between girls and boys (51). Furthermore, for all types of enamel defects, the published prevalence in the permanent dentition ranges from 2.2 % to 21.6 % (52, 53).

Although damage to the ameloblasts can occur due to a variety of agents, the abnormality in enamel is usually expressed in only a few ways:

- Hypoplasia, which is a reduction in quantity, presenting as pits, grooves, thin or missing enamel, or
- Hypomineralization, which is reduced mineralization presenting as soft enamel, or
- Hypomaturation where there is altered translucency affecting the entire tooth, or in a localized area known as an opacity (54).

In some cases, both hypoplasia and hypomineralization exist together and it may be difficult to differentiate between true hypoplasia and posteruptive breakdown of hypomineralized enamel (3). Hypoplastic enamel defects are thought to result from changes occurring during the stage of matrix formation whereas hypomineralization defects result from changes that affect the major part of the calcification process, and hypomaturation refers to the changes that occur at the last stages of mineral accumulation (55).

There are a number of factors that may affect the final appearance of the defect. These factors may include the stage of amelogenesis at which time the dysfunction occurs, the severity of the insult leading to temporary, or permanent inactivity of the cells, the duration of the insult, the phase of ameloblast activity during the relevant period, and the specific agent involved (55). The location of DDE depends on the stage of enamel production and on the time of the insult or injury to the ameloblasts. Furthermore, the location of DDE will vary in different homologous pairs of teeth as enamel production commences at varying times in different tooth types. All teeth at the same stage of development may be affected, with homologous pairs of teeth having similar types of DDE in similar locations. This type of distribution is referred to as generalized DDE and may be caused by systemic factors. When only one or

several adjacent teeth exhibit the same type of DDE, the defect-causing event is probably localized (56).

It is important to note that DDE with a similar appearance are not necessarily caused by similar aetiological agents. Conversely, the same aetiological factors can produce different defects at different stages of tooth development. In addition, enamel defects may also result from a combination of factors. It has been proposed that there are well over 90 different factors that may be responsible for causing DDE, but only a few of these factors have been confirmed as being directly responsible (57, 58).

PART II

MOLAR INCISOR HYPOMINERALIZATION

Definition

In 2001 Weerheijm and co-workers introduced the term Molar Incisor Hypomineralization (MIH) to describe the clinical presence of a qualitative enamel developmental defect of systemic origin that affects the FPMs and occasionally the incisors (9). This definition is now widely used and has been adapted by researchers and clinicians to describe MIH. However, based on the definition alone, it is not always possible to classify (diagnose) affected FPMs with MIH. Hypomineralization may be unrelated to MIH, one example being the diffuse opacities found in fluorotic enamel.

Prevalence

It would seem that MIH has become more readily seen and diagnosed, possibly as a result of the decline in caries rates (12). Due to the lack of an agreed definition, prior to 2001, the literature regarding MIH is confusing and it is difficult to be sure that different researchers are referring to the same condition. According to studies published after 2001, the prevalence of MIH ranges from 2.4% to 40.2% (Table 1). Most of the studies have been conducted in European countries, where the prevalence of MIH ranges from 3.6% to 25.0% (59). There are also reports from Asia, Australasia, Africa and South America. The wide range in prevalence reported in the different studies may reflect the real situation where different countries may have different prevalence. However, there is also a possibility that different assessment methods used in recording MIH, the differences between the examiners, the inclusion and exclusion criteria, and the conditions under which the examinations were conducted, have affected the reported prevalence from different studies. Furthermore, in 2010 Jalevik stated that comparison of the findings from various studies was difficult because of the use of different indices and criteria, examination variability, methods of recording and different age groups (10).

<i>Country</i>	<i>MIH prevalence</i>	<i>Study reference</i>
Australia	70% had ≥ 1 affected FPMs	Williams <i>et al</i> , 2006 ⁽⁶⁰⁾
Bosnia and Herzegovina	12.3%	Muratbegovic <i>et al</i> , 2007 ⁽⁶¹⁾
Brazil	40.2%	Soviero <i>et al</i> , 2010 ⁽⁶²⁾
Bulgaria	3.58%	Kukleva MP, Petrova SG, Kondeva VK, Nihtyanova TI, 2008 ⁽⁶³⁾
Denmark	15-25%	Esmark and Simonsen (1995) in Weerheijm and Mejare, 2003 ⁽⁵⁹⁾
Germany	5.9%	Preusser <i>et al</i> , 2007 ⁽⁶⁴⁾
Greece	10.2%	Lygidakis <i>et al</i> , 2008 ⁽⁶⁵⁾
Hong Kong	2.8%	Cho SY, Ki Y, Chu V, 2008 ⁽⁶⁶⁾
India	9.2%	Parikh <i>et al</i> , 2012 ⁽⁶⁷⁾
Iraq	21.5%	Ghanim <i>et al</i> , 2011 ⁽⁶⁸⁾
Italy	13.7%	Calderara <i>et al</i> , 2005 ⁽⁶⁹⁾
Jordan	17.6%	Zawaideh <i>et al</i> , 2011 ⁽⁷⁰⁾
Kenya	13.73%	Kemoli AM, 2008 ⁽⁷¹⁾
Libya	2.9%	Fteita <i>et al</i> , 2006 ⁽⁷²⁾
Lithuania	14.9%	Jasulaityte <i>et al</i> , 2007 ⁽⁷³⁾
Norway	13.9 %	Schmalfuss <i>et al</i> , 2016 ⁽⁷⁴⁾
Netherlands	9.7%	Weerheijm <i>et al</i> , 2001 ⁽⁸⁾
New Zealand	14.9%	Mahoney EK, Morrison DG, 2009 ⁽⁷⁵⁾
Sweden	18.4%	Jalevik <i>et al</i> , 2001 ⁽¹⁰⁾
	3.5%	Fagrell <i>et al</i> , 2011 ⁽¹³⁾
Turkey	14.8%	Alpoz and Ertugrul (1999) in Weerheijm and Mejare, 2003 ⁽⁵⁹⁾
UK	14.6%	Zagdwon <i>et al</i> , 2002 ⁽⁷⁶⁾

Table 1. The reported prevalence of MIH in different countries. Modified from Mishra et al, 2016⁽⁷⁷⁾.

Clinical findings

Clinically, MIH defects, which are qualitative defects classified as hypomineralized type, can be distinguished from carious lesions by their location on the teeth, and their colour, shape and hardness (78). These defects often follow incremental lines of enamel formation, from the cusps to the cemento-enamel junction (78, 79). Furthermore, MIH-affected teeth have different clinical presentations of enamel defects ranging from chalky white-opaque lesions with loss of translucency, to yellow/brown, atypical restorations, and/or with post-eruptive breakdown (80) (Fig. 7). The border between defective enamel and sound enamel is usually distinct making these defects well-demarcated (80). The cusp tips and the cervical enamel of MIH-affected teeth do not usually appear to be hypomineralized (81). Moving from the cervical level towards a more occlusal level, the defect is confined to the inner enamel while the outer enamel does not appear to be affected. The hypomineralized lesions become more evident towards the occlusal level and eventually spread through the entire thickness of the enamel (78).

The severity of MIH may vary greatly, ranging from mild opacities to posteruptive breakdown. It may be asymmetrical but in cases of severely affected FPMs the contralateral molar is more likely to be affected (82). In affected incisors, the severity of hypomineralization is usually less than that of the affected molars (83). There are also a few reports of the tips of permanent canines being affected as well as the second primary molars (84, 85). In addition, the distribution of the enamel defects in the mouth is usually asymmetrical; meaning that not all FPMs are necessarily affected to the same degree (80). The varying severities of enamel defects in permanent molars, incisors, and canines that develop at the same time suggest that not all teeth are equally sensitive to enamel defects and developmental disturbances (86).

Histological, chemical and mechanical findings

The histological appearance of MIH-affected teeth has been presented in the literature in a number of studies. The degree of porosity of the hypomineralized opaque areas varies from one tooth to another, and analyses have shown that yellow/brown enamel opacities are more porous than lighter-coloured opacities (87). The hypomineralized enamel extends from the enamel-dentine junction at a cuspal horn, usually around the mesio-buccal cusp (88), and it

may cover any area from the coronal third part to almost all of the enamel towards the enamel-cementum junction. Furthermore, enamel in teeth affected by MIH exhibits disorganized enamel prisms, a porous structure and loosely packed crystallites (89).

The mechanical properties, hardness, mineral density and modulus of elasticity of the hypomineralized enamel in MIH teeth have lower values compared with normal enamel (55, 88, 90). The lower values may explain why the enamel surface on a tooth with MIH often collapses and fractures under occlusal load. Moreover, elemental analyses of MIH-enamel have revealed some changes in the chemical composition and a reduction in the mineral composition (88, 91). A correlation between hardness values, mineral density and the colour of the hypomineralized enamel has also been shown, with yellow/brown opacities being softer than white opacities (55). On average, the mineral density in MIH-affected enamel is about 19% lower than in sound enamel (4, 92). The calcium : phosphorus (Ca:P) ratio in MIH-enamel was found to be lower than in adjacent normal enamel (1.8) (91). The fluoride content has been shown to be highly variable, the sodium content is higher towards the surface of the hypomineralized enamel, there is a higher magnesium and potassium content, and a negligible difference in the chlorine and strontium content (3).



Figure 7. Examples of affected FPMs from three individuals with MIH. Defects vary in severity from diffuse yellow opacities (black arrow) in tooth 16 (a) to more severe changes due to posteruptive breakage (PEB) in tooth 46 (b) and atypical filling due to severe MIH in tooth 26 (c). (The figures are copied from Sidaly R, Schmalfluss A, Skaare AB, Sehic A, Stiris T, Espelid I. Five-minute Apgar score \leq 5 and Molar Incisor Hypomineralisation (MIH) - a case control study. *BMC Oral Health*. 2016;17(1):25)

Diagnosis & differential diagnosis

It is important to diagnose MIH, differentiating it from other developmental disturbances of enamel. The diagnosis of MIH is usually made clinically and, in order to diagnose MIH, at least one of the FPMs has to be affected. As mentioned previously, the defects can also be seen in second primary molars, incisors and the tip of canines. If there are several affected molars and incisors, the defects could be considered to be more severe. The age of 8 years is optimal for detection of MIH, as at this age all permanent first molars and most of the incisors are erupted. In addition, the permanent first molar teeth will usually be in a relatively good condition without excessive posteruptive breakdown and with fewer restorations due to MIH and caries.

Regardless of aetiology, teeth with DDE may present similarly, allowing the development defects of enamel hypoplasia to be easily confused with MIH. In cases of MIH-affected molars with posteruptive enamel breakdown (PEB) due to caries or masticatory trauma, it may be especially difficult to differentiate between MIH and enamel hypomineralization. Moreover, MIH can be masked by extensive caries or restorations in a child with high caries rate. PEB may lead to a clinical picture resembling hypoplasia. However, in hypoplasia, the borders of the deficient enamel are smooth, while in posteruptive enamel breakdown the borders to normal enamel are usually irregular (93). MIH can also be confused with fluorosis, however, the enamel opacities of fluorosis are diffuse, in contrast to the normally well-demarcated borders of hypomineralized enamel seen in MIH (93). In addition, fluorosed enamel is generally caries resistant, in contrast to the caries prone MIH-affected enamel. Furthermore, the difference between MIH and AI is one of definition. In most cases, MIH produces asymmetrical disorders in FPM's and incisors, unlike AI in which all teeth are affected symmetrically. MIH is also a chronological condition affecting teeth from the same period in tooth development. Furthermore, there is usually a positive family history in cases of AI (93, 94).

Clinical implications

MIH presents a set of problem that may require a multi-disciplinary approach for management. Depending on the severity patients affected by MIH may present several clinical problems, including rapid wear, enamel loss, increased susceptibility to caries, sensitivity, and dental

fear and anxiety associated with pain (95). Due to the fact that MIH-affected enamel is less dense and may be porous and discoloured, some MIH-affected teeth are subject to post-eruptive breakdown, and have greater sensitivity to temperature changes or mechanical stimuli (80, 96, 97). Due to this greater sensitivity, a simple procedure such as brushing the teeth may be difficult for children with MIH, even when the enamel is not broken down. Children may avoid brushing because of sensitivity and caries progression may therefore be very rapid in these children. When there is loss of tooth substance due to post-eruptive breakdown they usually experience severe discomfort during eating and tooth brushing, which increases the risk of plaque accumulation and dental caries, and further compromises the affected teeth and gingival status (80). It is suggested that the aetiology of the increased sensitivity is physiological, based on repeated small pain stimuli from subclinical pulpal inflammation due to the increased porosity of the enamel (93). In a study comparing the pulps of non-carious hypomineralized FPMs to those of apparently sound FPMs from MIH affected individuals, it was concluded that the differences were indicative of inflammatory changes (98).

MIH-type enamel alterations pose a great challenge not only for the patients, but also for the dentist. Hypomineralized teeth have been shown to be difficult to anaesthetize, and this can result in discomfort for the child during dental treatment (96). It has been recommended to supplement local anesthesia with sedation or relative analgesia when local anesthesia alone does not work (60, 99). It may also be useful to use systemic pain control for restorative care in these patients. Due to the fact that MIH becomes apparent only with the eruption of the permanent first molars and permanent incisors, significant dental treatment may be required at the ages of six to eight years. In such a young age group, this can be a challenge and may lead to dental anxiety (80, 96).

Children with MIH may also experience poor aesthetics when anterior teeth are involved (100). It is therefore important that the aesthetic aspect is also taken into account during treatment planning. Apart from the restorative difficulties faced by dental clinicians, children with MIH have been reported to have higher levels of dental fear and anxiety. Further, children with MIH have been shown to receive more dental treatment than unaffected children

(101). Several studies have shown that MIH molars require more visits, more invasive and expensive treatment, and higher restoration failure rates are demonstrated (96, 97, 102).

Clinical management

MIH's clinical management is challenging mostly due to the limited cooperation of a young child, but also difficulty in achieving anesthesia and repeated marginal breakdown of restorations make the treatment challenging. The available treatment modalities for teeth with MIH are extensive, ranging from prevention, to restoration, and lastly to extraction. However, multifactorial treatment concept is usually required in order to deal with the different aspects of the disease. The decision on which treatment should be given is complex and is dependent upon on a number of factors, including the severity of the condition, the patient's dental age and the child/parent's social background and expectation. Furthermore, it is recommended that the management of MIH focuses on the three aspects - controlling the sensitivity, short-term management, and long-term management. Although, there is no universal classification for MIH, Alaluusua and co-workers have classified the hypomineralized areas as mild (colour change: white, yellow or brown), moderate (loss of enamel only) and severe (loss of enamel in association with affected dentine) (103). This classification can be indicative of how clinicians manage MIH-affected teeth.

There is a great variability between the treatment decisions dentists make for MIH-affected molars, however, there is a general consensus that fissure sealants are the treatment of choice in 'mildly' affected cases, where the enamel appears to be of good quality and clinical and radiographic investigations have confirmed that the molar is caries free (104). However, post-eruptive enamel breakdown remains a risk, and a composite restoration may be necessary if breakdown occurs. This is the reason for why it is important that even mild cases are checked regularly, at least two to three times annually (100). In 'moderate' cases, where the enamel/dentine defect (PEB) is well demarcated and confined to one or two surfaces, composite restoration is the treatment of choice. However, further breakdown of affected enamel can occur at the restoration margins, which may mean that a different treatment procedure, e.g., stainless steel crown, will be required later. In 'severe' cases of MIH, where there is frequently cuspal fracture, with or without pulpal involvement, the treatment options are either restoration, i.e. full molar crown coverage (stainless steel crowns), or tooth

extraction. A combined endodontic-orthodontic opinion is essential in such cases (104). Factors such as the occlusion, presence or absence of crowding, overall dental development, missing or malformed teeth, and long-term prognosis will determine the decision to retain or extract the affected molars. The timing of extraction of FPMs is now less critical with the extensive use and availability of orthodontic fixed appliances (105). Symptomatic molars may pose a difficulty in ensuring that extractions are carried out at the optimum time. In such cases, a glass ionomer material can be used as an interim restoration to help resolve symptoms (106).

The treatment of the affected incisors in MIH will be determined by the severity of the condition, as the severity of incisor hypomineralization is usually less than that of the affected molars (80). Affected incisors rarely exhibit post-eruptive breakdown since they are not subjected to the heavy occlusal loading sustained by FPMs, and aesthetic considerations are the prime factors when planning intervention in such cases. The incisal opaque defects usually extend through the full thickness of enamel, meaning that acid/pumice micro-abrasion techniques used alone tend to produce little improvement in appearance (100). Bleaching may improve yellow brown discolourations but is unlikely to improve the underlying opacity (106). Unsightly opacities and defects on permanent incisors of young children can be successfully masked using direct composite veneers (107), however, it may be necessary to replace these composite veneers with porcelain veneers to obtain a satisfactory aesthetic result.

PART III

AETIOLOGICAL CONSIDERATIONS

There is little knowledge and understanding of the structure and biochemistry of MIH affected teeth. Therefore, by understanding its causes and aetiology, it is reasonable to anticipate that MIH may be managed better in the future. MIH origins are subject to controversy in scientific literature and its exact pathogenesis is still unknown. In the literature various causes for MIH have been suggested, however, in line with general medicine pathologies, the most common hypothesis is that it may result from a local malfunction during enamel formation, taking place at the embryonic stage and during early childhood (93). This section reviews the literature surrounding prenatal, perinatal and postnatal factors associated with MIH aetiology.

The mineralization of the primary teeth starts at 14-18 weeks in utero. The formation of the primary tooth roots is completed between 1.5 and 3 years. The crowns are halfway mineralized at birth and become fully formed during the first year of life. Mineralization of the permanent teeth starts approximately at birth, beginning with the first molars. The incisors and canines start their mineralization during the first year of life, whereas mineralization of the premolars and second molars starts between the second and third years of life. However, the normal time range is wide. The crowns of the permanent teeth (except third molars) are generally completed between 5 and 7 years of age. In general, the mandibular teeth develop earlier than the maxillary teeth. A marked sex difference has been observed in tooth formation, girls being on average half a year ahead of boys (108).

Prenatal factors

The prenatal period begins with fertilization of the oocyte and ends with delivery, thus the period in utero until 36–38 weeks of fetal development. Although aetiological factors acting during this period produce fewer cases of MIH compared with all other periods, indicating that fetus is probably protected in utero (65), studies have reported that there is some evidence that medical problems during pregnancy are associated with MIH (65, 109). A study from 1953 has shown that maternal pyrexia has a detrimental influence on amelogenesis, ranging from ameloblastic dysfunction to complete cellular degeneration (110). It has been reported that medical problems during pregnancy are more common in mothers of MIH-children than in mothers whose children do not have MIH (65, 111). These problems included rhesus

disease, hypertension and pre-eclampsia. Furthermore, maternal diabetes, which produces hypocalcaemia in the mother and oxygen shortage problems to the infant (112), may result in enamel hypomineralization (65). Moreover, a study by Fredén and co-workers showed that urinary infection during the last trimester was associated with MIH-like lesions (113).

A recent study reported that MIH was more common in children whose mothers suffered from prenatal illness requiring hospitalization and long-term illness during pregnancy (114). However, it has been reported that the use of medication to avoid a premature birth, and the use of paracetamol/acetaminophen, during pregnancy, was not associated with MIH (115). These findings differ from data reported in a previous study, which found that prenatal conditions were significantly more likely to be associated with the development of MIH (116). Jalevik and co-workers reported in 2001 that the calcium content was very low in MIH lesions, suggesting that they were caused by impaired calcium metabolism of the ameloblasts (117). Several studies have therefore investigated the role of hypocalcaemia, an abnormal condition that may occur in the perinatal period but also in prenatal and postnatal periods, in MIH aetiology (11, 65, 82). Hypocalcaemia can be associated with several conditions such as maternal diabetes, vitamin D deficiency during the prenatal and/or perinatal period and prematurity. In a prospective study it was found that MIH-like lesions and enamel hypoplasia were significantly more common in premature infants than in controls (118). However, results of experimental studies on the effects of hypocalcaemia on developing dental hard tissues seem to be related to the duration of hypocalcaemia.

Perinatal factors

Several studies have reported that in the perinatal period, the period between delivery and up to the first month following birth, different medical conditions alone (or in combination) affecting a child may be associated with the occurrence of MIH. In a Greek study by Lygidakis, MIH was more frequently seen in the case group, where the most common perinatal problems/conditions were caesarean section, prolonged delivery, premature birth or twinning (83). A Swedish study by Brogårdh-Roth and co-workers demonstrated that MIH was more common in children born preterm than in children who were born at term (119). However, other studies have failed to demonstrate any relationship between MIH and

prematurity (61, 120). In addition, perinatal medical conditions appear to be associated with hypocalcemia and hypoxia. Previous studies have shown that early neonatal hypocalcemia is present in approximately 30 to 75% of cases of preterm low birth neonates, particularly in those with respiratory distress and birth asphyxia due to complicated, prolonged or difficult delivery (82, 121). This can be explained by the fact that two-thirds of an child's stores of calcium and phosphorus accumulate during the last trimester of pregnancy and preterm infants miss much of this mineral accretion (122).

Hypoxia, a pathological condition in which the body is deprived of adequate oxygen supply, can be associated with birth problems such as prematurity, respiratory distress and excessively prolonged duration of labour. Respiratory distress is a syndrome in premature infants caused by developmental insufficiency of surfactant production and structural immaturity in the lungs. As the disease progresses, the baby may develop ventilatory failure (rising carbon dioxide concentration in the blood) leading to lack of oxygen supply to tissues. Results from several studies have shown that a lack of oxygen in active ameloblasts could be the causative factor of MIH or opacities in molars and incisors (7, 65, 118). Furthermore, newborns delivered by elective cesarean section at around full-term have been shown to have an increased risk of overall and serious respiratory illnesses, conditions often associated with hypoxia (123). Also, the commonly used spinal anesthesia for cesarean section has a frequent complication of maternal hypotension that can be associated with severe nausea or vomiting which occasionally produces infant hypoxia (121). Maternal hypoxia during the latter stage of pregnancy has been demonstrated to disturb amelogenesis in the rat foetus (124), and animal studies have shown that hypoxia caused enamel hypomineralization in rat and mice incisors (125, 126). However, a study performed on children with low Apgar score, which is known to be indicative of hypoxia during labour, showed no statistically significant difference regarding the presence of MIH (127).

Postnatal factors

Several studies have suggested that medical problems in the postnatal period, the period from 29 days to 4 years of age, are associated with MIH (11, 65, 115). Although medical conditions in the first three years of life has been the most discussed aetiological factor associated with

MIH, the findings from several studies are contradictory and the results are inconclusive. Childhood illnesses reported in the literature included otitis media (120), upper and lower respiratory tract infections (120, 128), asthma (128), episodes of high fever, chicken pox (111) and urinary tract infections (129). One study demonstrated that more of those with MIH had had illnesses during the first four years of life than those without MIH (120). This was also the case for another study by Jalevik and co-workers in 8-year-old Swedish children that reported an association between diseases at 0 to 1 year of age and subsequent MIH (128). This was consistent with the results from a study by Chawla and co-workers, where children with MIH showed histories of medical conditions that may be linked to MIH (102). However, no control group was used in that study, and direct comparison to healthy children could not be made.

On the other hand, a study performed on twelve-year old children in Bosnia and Herzegovina did not demonstrate any significant associations between MIH and common childhood illnesses (including urinary tract infections, otitis media, high fever, asthma, bronchitis, pneumonia, tonsillitis or the use of antibiotics), either separately or in combination (61). A similar study in German children by Dietrich and co-workers had similar findings (130). Another similar study in a group of UK children by Whatling and Fearne included all the previously mentioned childhood illnesses as well as other illnesses, vaccinations and allergies, and found only that the occurrence of chicken pox showed a significant association with the occurrence of MIH (111). The possible role of vitamin D deficiency and rickets in the aetiology of MIH has also been investigated, because mild vitamin D deficiency may cause enamel hypomineralization without the classical signs of rickets. However, the study found no relationship between vitamin deficiency and MIH (130). Similar findings were reported in a matched case-control study in Bosnian children (61).

There are only a few drugs that have been recognized to disturb dental hard tissue formation. Among them are anticancer drugs, such as cyclophosphamide, and the tetracyclines, which cause discolouration of developing teeth (131). It has also been suggested that the use of antibiotics is associated with MIH (79, 120, 128), however the possible association of MIH with antibiotic use is still based on weak evidence. In a survey of 141 children, Laisi and co-

workers found that only 23 children (16.3%) had MIH and it was found that MIH was more common among those who had taken amoxicillin or erythromycin during the first year of life (132). No association with other antibiotics was found. The possibility that other confounding factors (such as the infections or fevers) may be related was not assessed. In a UK study, it was found that MIH was more common among children who received only amoxicillin during the first four years of life, but not in children who received mixed antibiotics including amoxicillin (79). Tapias-Ledesma and co-workers (129) and Muratbegovic and co-workers (61) did not find any association between any type of antibiotics and MIH. None of the studies have proved that the childhood infections (and associated fever) or the treatment with an antibiotic are causative factors. The association with amoxicillin is probably more likely to have been because of its very common use for childhood infections. It is therefore more likely to be the infections (and associated fever) themselves that are the cause, as demonstrated in a study by Suckling (51, 133).

A recent review by Silva and co-workers concluded that childhood illness is likely to be associated with MIH (134). Ameloblast function is highly sensitive to changes in the local surrounding environment, including changes induced by systemic illness (135). The authors suggested several mechanisms for how such factors lead to the specific changes in ameloblasts that then results in MIH. One of the mechanisms was that prenatal exposure to endocrine disrupting chemicals (EDCs) can result in MIH-like lesions by increasing the expression of enamel proteins, reducing the expression of the kallikrein four gene and leading to the accumulation of albumin, which hampers crystal growth. However, there are no observational studies investigating this link (136). The possible relationship between fever and enamel defects has also been explained by altered expression of genes important in enamel formation (137). However, the authors concluded that further laboratory studies regarding the protein composition, structure and ultrastructure of hypomineralized enamel will compliment observational studies in establishing a clear pathogenesis for MIH (134).

Genetic factors

It is well known that the amelogenesis phase of the enamel development is under strict genetic control, and that the size, shape, shade, and even enamel microhardness can be affected by genetic variation (15, 27). Different kinds of enamel defects may occur depending on the

stage of development affected. Very few studies have investigated genetic or ethnic factors in the aetiology of MIH, however, considering that the entire enamel formation process is under genetic control, it is reasonable to hypothesize that genetic variations could be associated with alterations in the amelogenesis. Whatling and Fearnese seem to agree that there may be genetic predisposition associated with one or more of a range of systemic insults occurring at a susceptible stage in the development of specific teeth, and suggested that family studies may provide further information (111). Because the development of teeth occurs over time, insults at different times may also cause different forms of this problem. Mahoney and Morrison found no statistically significant ethnic differences in MIH prevalence among the Maori, Pacific Island, and New Zealand European ethnic groups (75).

Vieira and co-workers was the first to suggest that MIH has a genetic component that involves genetic variation in genes expressed during dental enamel formation. In 2003 they investigated whether genetic variation in enamel formation genes is associated with MIH by testing DNA samples from cases with MIH and unaffected controls (138). The study suggested trends for association between hypomineralized enamel and genetic variation in enamel formation genes. It was possible to note that some variants indicated a trend for association with susceptibility to MIH, while others showed a trend in the other direction, suggesting a protective effect against the development of MIH. Interestingly, no association was found with regard to AMELX, a fundamental gene that secretes the main protein of dental enamel, the amelogenin, during the secretion stage of amelogenesis. The authors suggested that the enamel defect in MIH does not seem to be directly related to the function of amelogenin during the secretion stage of amelogenesis (138). However, they found evidence for a trend of association between variation in the TUFT1 and TFIP11 genes and MIH, which suggests that these genes are potentially involved in individual predisposition to MIH.

Recently, Vieira & Kup proposed that MIH is a genetic condition related to disturbances in the maturation stages of enamel, which in most instances is localized to FPMs and incisors but occasionally also affects the second primary molars and permanent canines and premolars (138). The involvement of additional teeth may be due to the influence of additional gene variants in any of the more than 100 genes expressed during late enamel development (139).

In a family-based genetic association study investigating the genetic carriage potentially involved in MIH development, it was reported that variations in genes related to amelogenesis were associated with the susceptibility to develop MIH (140). The genes included FAM83H, AMBN, Bone Morphogenetic Protein 2, 4, 7 (BMP2, BMP4, BMP7), ENAM, MMP20, Distal-less-homeobox3 (DLX3), Fibroblast Growth Factor Receptor1 (FGFR1) and AMELX gene. The authors concluded that this result was in agreement with the multifactorial idea of the MIH aetiology, but further studies are necessary to more thoroughly investigate the factors that could influence MIH.

PART IV

SUMMARY

MIH affects a substantial number of children worldwide and impacts greatly on treatment need and dental anxiety, and there are many studies, but the aetiology is still uncertain. The majority of existing studies imply that the aetiology of MIH is complex with undetermined systemic and genetic factors disrupting normal amelogenesis in the affected teeth. In an attempt to explain the possible aetiological factors it is important to remember that between 28 weeks in utero and the first 10 days of life, ameloblasts initiate amelogenesis in the first permanent molars, followed later on by the other permanent teeth (82). Interruption in the function of the ameloblasts, temporarily or permanently, and depending upon the time of insult, will produce enamel hypoplasia or enamel hypomineralization.

Many retrospective, cohort and case-control studies have examined a wide variety of putative possible causes for MIH (12, 60). Factors associated with disrupted amelogenesis, including systemic conditions and environmental insults influencing natal and early development of FPMs, have been studied (65, 111, 120, 141, 142). The last trimester of pregnancy is a critical period during which the amelogenesis of FPMs and incisors teeth starts. Multiple episodes of maternal high fever, viral infections such as rubella and chickenpox, prolonged vomiting up to the last month of pregnancy, urinary tract infections, maternal hypertension, maternal diabetes, renal deficiency, and malnutrition during the last trimester of pregnancy, are some of the presumed causative factors (12, 75, 111). However, fewer cases of MIH were related to illnesses in the prenatal period, compared with all other periods, indicating that the developing foetus may to some extent be protected in utero from putative risk factors (119).

Studies have shown that in the perinatal period different medical conditions, alone or in combination, may affect the welfare of a child (11, 12). The most common reported perinatal problems associated with MIH are caesarian section, prolonged/complicated delivery, premature birth and twinning. Hypoxia, low birth weight, hemorrhage and detachment during delivery are other supposed perinatal causes for defective ameloblast function (65). However, special attention has been paid to the possible association between medical problems in the postnatal period and MIH. These problems may include prolonged childhood illnesses, prolonged high fever due to infections, repeated/prolonged medications (antibiotics like

amoxicillin) and exposure to environmental contaminants (65). Experiments have shown that conditions affecting the enamel matrix pH, i.e. respiratory acidosis and abnormal oxygen levels resulting from hypoventilation in various respiratory diseases, inhibit the action of the proteolytic enzymes and the development of the crystal hydroxyapatite, thus resulting in enamel hypomineralization (82). However, further prospective studies are needed to investigate the role of medications, and whether the relationship changes with the type, number and severity of illnesses.

Although researchers have for several years speculated on the aetiological factors involved in the development of MIH, the retrospective nature of most MIH studies is a major problem. These studies rely mainly on parent recall, often many years after the event, and the strength of the evidence is therefore limited. Furthermore, not all information obtained in such studies are trustworthy, as some aspects of maternal health during pregnancy, recall of breastfeeding duration, child illness and medication use are less likely to be reliable (143). Although there is a need for prospective studies to improve the level and strength of evidence of the role of the present putative factors and to reveal new factors that may be involved in the aetiology of MIH, there are many barriers to conducting such studies, including cost, loss to follow-up and non-participation. As such, using existing medical cohorts provide a practical means of conducting prospective studies.

The aetiology of MIH remains unclear, and it is impossible at this time to label any one factor as being an aetiological one because of non-specific, weak and conflicting reports about the aetiology of MIH. Generally, almost all the studies that have explored the possible aetiological factors behind MIH have agreed that there is no strong and valid support for any one particular aetiological factor(s) (12, 97, 99, 111, 144). MIH may have a multifactorial aetiology, with factors acting together or even synergistically (11-13). There may be different types of MIH, and there may be a genetic predisposition associated with one or more of a range of systemic insults occurring at a susceptible stage in the development of specific teeth. Therefore, current knowledge suggests that MIH may have a multifactor aetiology acting additionally or even synergistically. As well as, a genetic predisposition associated with one or more of a range of systemic insults occurring at a susceptible stage in the development of

specific teeth. This explains why in a seeming random manner several teeth can be severely affected while their bilaterally teeth are unaffected.

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