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WHAT DOES TOOTH ENAMEL TELL US OF LIVED LIVES?

A study of the neonatal line and other accentuated lines in human deciduous enamel

Jaana Hurnanen

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Dedicated to my mother for her statement
"If only I could still experience the day of your defence"
and all the "young researchers" starting their career not so young

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Faculty of Medicine
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ABSTRACT

The purpose of this study was to investigate the varying width and structure of the neonatal line (NNL) and the occurrence of other accentuated lines (ALs) and their association with known early life events. The NNL was identified and the effect of the mode and duration of delivery on the NNL width was assessed (Study I). The effect of tooth type and perinatal factors on the formation of the staircase (Sc) pattern in the NNL was evaluated (Study II). The probabilities were analysed for how likely certain life events are determinants of ALs (Study III).

The 129 investigated Finnish Deciduous Tooth (FDT) collection teeth are part of a longitudinal population-based follow-up study, the Finnish Family Competence study. The NNL was detected, and the width measured using a transmitted light microscope (Study I) and the Sc pattern was investigated using polarisation (Study II). ALs were identified from polarisation light microscope tile scan images and compared with known postnatal life events (Study III).

The NNL was identified, and width was inversely associated with the duration of delivery ($p=0.0097$). The mode of delivery had no effect on the NNL width (Study I). The Sc pattern was highly related to the tooth type ($p<0.0001$) and more present in the in the middle third of the vertical crown wall enamel ($p<0.0001$). Mode, duration of delivery or pain medication did not affect the NNL Sc pattern formation (Study II). 56% of canine ALs were located in the middle third of the crown height. Vaccinations and/or ear infections were not regular determinants for ALs (Study III).

The neonatal line exists in practically all deciduous teeth. Its width is inversely interrelated with the duration of delivery. The neonatal line structure and the occurrence of accentuated lines are dependent on their topographical location: in the mid-height of the tooth crown the ameloblast susceptibility seems stronger along the enamel forming front. The most frequently appearing disease, ear infection, and vaccinations are not likely to always engender accentuated lines in the FDT collection samples.

KEYWORDS: accentuated lines, deciduous tooth, tooth enamel, neonatal line, population study

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TIIVISTELMÄ

Tutkimuksen tavoitteena oli tutkia neonataalilinjan (NNL) vaihtelevaa leveyttä ja struktuuria sekä muiden korostuneiden linjojen esiintyvyyttä ja niiden yhteyttä tiedettyihin elämäntapahtumiin. NNL identifioitiin ja arvioitiin synnytystavan ja -keston vaikutusta NNL:n leveyteen (Osatyö I). Tutkittiin hammastyypin ja syntymänaikaisten tekijöiden vaikutusta NNL:n porrasmuodon syntyyn (Osatyö II). Analysoitiin todennäköisyyksiä, kuinka tietyt elämäntapahtumat mahdollisesti aiheuttavat kiilteen korostuneen linjan (Osatyö III).

Tutkitut 129 Finnish Deciduous Teeth (FDT) -kokoelman hammasta ovat osa pitkäaikaista populaation seurantatutkimusta, Finnish Family Competence tutkimusta. Läpivalaisumikroskoopilla havaittiin NNL, ja sen leveys mitattiin (Osatyö I) ja porrasmuoto tutkittiin polarisaatiolla (Osatyö II). Polarisaatiomikroskooppi-kuvayhdistelmistä identifioitiin korostuneet linjat, joita verrattiin syntymän jälkeisiin rekisteröityihin elämäntapahtumiin (Osatyö III).

NNL identifioitiin ja leveys oli käänteisesti verrannollinen synnytyksen kestoon ($p=0097$). Synnytystavalla ei ollut vaikutusta NNL:n leveyteen (Osatyö I). NNL:n porrasmuoto liittyi merkittävästi hammastyyppiin ($p<0.0001$) ja oli eniten esillä kiilleseinämän vertikaalisessa keskikolmanneksessa ($p<000.1$). Synnytyksen tyyppi tai kesto tai käytetty kivunlievitys eivät vaikuttaneet porrasmuodon muodostumiseen (Osatyö II). Kulmahampaissa 56 % häiriölinjoista sijaitsi kruunun korkeuden keskikolmanneksessa. Rokotukset ja/tai korvatulehdukset eivät säännönmukaisesti aiheuttaneet korostuneita linjoja (Osatyö III).

Neonataalilinja esiintyy käytännöllisesti katsoen kaikissa maitohampaissa. Sen leveys on käänteisesti yhteydessä synnytyksen kestoon. Neonataalilinjan struktuuri sekä korostuneiden linjojen esiintyvyys ovat riippuvaisia topografisesta sijainnista: kruunun keskikorkeudessa ameloblastien herkkyys vaikuttaa vahvemmalta kiilteen kehitysrintamassa. Tavallisin sairaus, korvatulehdus, tai rokotukset eivät näytä aina synnyttävän korostuneita linjoja maitohampaissa.

AVAINSANAT: hammaskiille, korostuneet linjat, maitohammas, neonataalilinja, populaatiotutkimus

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Abbreviations

AL	accentuated line
BSE	back-scattered electron
bsR	brown striae of Retzius
CFT	crown formation time
EDJ	enamel-dentin junction
FDT	Finnish deciduous tooth
FFC	Finnish family competence
NNL	neonatal line
POLMI	polarisation light microscope
Sc	staircase
SE	secondary electron
SEM	scanning electron microscope

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Hurnanen, J., Visnapuu V., Sillanpää, M., Löyttyniemi, E., Rautava, J. (2017). Deciduous neonatal line: Width is associated with duration of delivery. Forensic Science International 271, 87-91. https://dx.doi.org/10.1016/j.forsciint.2016.12.016

II Hurnanen, J., Sillanpää M., Mattila, M-L., Löyttyniemi, E., Witzel, C., Rautava, J. (2019). Staircase-pattern neonatal line in human deciduous teeth is associated with tooth type. Archives of Oral Biology 104, 1-6. https://doi.org/10.1016/j.archoralbio.2019.05.016

III Hurnanen, J., Sillanpää, M., Reinhold, V., Löyttyniemi, E., Rautava, J. (2022). Reflections of life: Association of enamel accentuated lines with early life events in modern Finnish deciduous teeth. Submitted.

1 Introduction

Tooth enamel is at best a mirror for human life and for physical abnormalities occurring during the years of enamel formation. However, this quality has been seldom utilised in medical applications, not even for deciduous teeth. Human tooth enamel possesses features underlying a possibility to later investigate and date circumstances that occur during perinatal, early life and adolescent development.

Diseases and traumas may affect enamel-forming cells and engender microstructural disturbances called accentuated lines (ALs) in developing enamel (Rose et al., 1977; FitzGerald et al., 2006; Schwartz et al., 2014). The disturbances remain to be later investigated because mineralised enamel does not change or repair itself. ALs form throughout enamel development, during the time period from about the 16th gestational week until the wisdom teeth enamel is formed. When an individual undergoes stress, the phenomenon hits all the enamel-forming cells in all the developing teeth at that time.

Seminal studies by Rushton (1933) and Schour (1939) introduced one disturbed line, the neonatal line (NNL), found in deciduous and the first permanent molar teeth. This line expresses the time of a child’s birth, a chronological point zero in enamel development. In the first deciduous tooth, the enamel development commences approximately 20 weeks before birth. Gradually, still in utero, all the deciduous teeth and most often one cusp in the first permanent molars start their enamel development, which in deciduous teeth continue approximately until the end of the child’s first year of life (Birch & Dean, 2014). The NNL is found in each deciduous tooth type, in location, related to its stage of enamel development at the time of birth. The child’s pre-, at- or post-term status (Skinner & Dupras, 1993) also affects the location. The NNL creates a possibility to separate pre- and postnatal enamel.

The causes behind other ALs are acknowledged to be stressors like diseases and traumas. Enamel possesses a most applicable feature by growing in a circadian rhythm (Boyde, 1989). The daily layers in enamel can be counted and this enables the chronological timing of the recorded postnatal ALs in relation to NNL, the day zero. When comprehensive clinical histories are available, ALs have been matched to certain single life events (Schwartz et al., 2006; Birch & Dean, 2014).

Published results of the NLL characteristics as well as life event related ALs are not excessive. We had an opportunity to use a collection of deciduous teeth with attendant early life medical data, a part of a longitudinal population-based study called the Finnish Family Competence study. This offered a possibility to supplement research on the field of the NNL and ALs in human deciduous tooth enamel.

2 Review of the Literature

2.1 Development of deciduous teeth

All humans have two sets of teeth: primary dentition, also called deciduous teeth, and permanent dentition. A tooth comprises of three hard tissue layers: 1) enamel, the outermost hard mineralised 1-2 mm thick layer covering the tooth crown, 2) dentin, inside the crown and the root, and 3) cement, thinly coating the root. Dental pulp tissue is found in the root canal.

The deciduous teeth already start to develop in utero and are fully developed at approximately the age of three years. The change from primary to permanent dentition occurs gradually, between the ages of six to 12 years. Permanent teeth grow inside the alveolar bone and while developing and erupting into the oral cavity, they resorb the roots of the deciduous teeth, until only the deciduous crowns are attached in the soft tissue and eventually become loose. By the age of about 12 years, all the primary teeth have been replaced by permanent ones. (AlQahtani & Liversidge, 2010).

The development of a tooth arises from two different embryonic tissue layers, the epithelium and the underlying mesenchyme. From the 6th to 10th gestational week, the tissue layers go through bud- and cap stages, where moulding of cells eventually generates 20 enamel organs, the sources of the future deciduous teeth (Fig. 1). As enamel organs for deciduous teeth develop further, approximately in the 16th gestation week, the initiation of enamel organs for permanent teeth commences (Hillson, 2002).

The enamel organ originates from the epithelial layer and forms a cap-like structure. The mesenchymal cells form the dental papilla inside the cap, and a dental follicle underneath and outside the cap. Enamel organ cells differentiate to form an internal enamel epithelium, which will eventually secrete enamel matrix to be mineralised into mature enamel. The dental papilla inside the cap will ultimately form dentine and the dental follicle forms cement (Fig. 1) (Hillson, 2002).

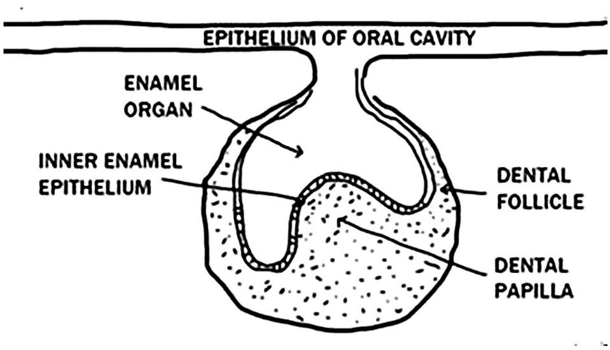

Figure 1. Enamel organ (own drawing).

The enamel organ continues to grow into the bell stage, and the inner enamel epithelium cells divide, moulding themselves roughly into the form of each distinctive tooth type. When the division is finished, the shape of the inner enamel epithelium equals the form of the enamel-dentin junction (EDJ) that separates enamel and dentin in a fully formed tooth. At this point, a series of events occur that trigger one another: the differentiation of the first ameloblasts, the enamel-forming cells, from the inner enamel epithelium initiates the differentiation of odontoblasts, the dentin-forming cells, from mesenchymal cells. The odontoblasts then secrete the first layers of predentin which in turn sets off the secretion of the enamel matrix by the ameloblasts (Hillson, 2014).

Both dentitions, when developing, go through the same formation pattern in a distinctive order. The tooth crown formation in the incisors starts from the incisal tip and in premolars and molars from the cusp tips. Layers of enamel build up outwards from the EDJ on top of each other until the incisal edge or the occlusal part of the premolars and molars is composed. Parallel to the cervical development of the enamel, dentin grows inside the tooth crown simultaneously. The crown enamel walls are formed as the layers increment, and at the same time gradually proceed downwards, only partially covering the layer underneath, till the most cervical rim has been completed (Hillson 2002).

Humans' teeth are divided into two quadrants in the upper jaw, the maxilla and two quadrants in the lower jaw, the mandible. In each quadrant, two deciduous incisors will first develop, followed by a deciduous canine and finally two deciduous molars (Hillson, 2002). At the time of birth, the deciduous teeth have not yet erupted into the mouth, but the beginnings of all 20 deciduous teeth are visible in a radiograph (AlQahtani & Liversidge, 2010).

2.1.1 The neonatal line

Rushton (1933), when investigating ground sections of deciduous teeth, introduced a fine contour line, which was "well-marked and always in about the same position in any particular type of tooth". He further stated that "the amount of enamel then formed tallies well with that known to be formed by the time of birth". He concluded that this line, the birth-ring, represents the effect of the event of birth. Schour (1936), studying hundreds of teeth and noticing a distinctive incremental line positioned in the same location in each deciduous tooth type, suggested that "some constant and universal condition causes this phenomenon". The incremental line formed at birth or soon after was named the neonatal line (NNL) by Schour.

2.1.2 Location of the neonatal line

The NNL is found in ground sections of all deciduous teeth and in the mesiobuccal cusp of the first permanent molar of a child born alive, because these teeth are developing during birth (AlQahtani & Liversidge, 2010). In a mesiobuccal cusp of the first permanent molar, the NNL line can disappear because of the scarcity of prenatal incremental layers (Antoine et al., 2009). In the first permanent molars, to get the NNL visible, an ideal and exact plane of sectioning is required, accurately through the cusp and underlying dentin horn, not to lose any prenatal enamel. The NNL is also known to exist in dentin (Schour, 1936), as odontoblast activity is similarly disrupted during parturition. To our knowledge, however, previous research is limited to the enamel NNL.

The location of the NNL inside the tooth crown enamel varies in different tooth types, marking the frontline where ameloblasts on that tooth were proceeding at the time of birth (Schour, 1936) (Fig. 2). The gestation time dictates the location of the NNL inside the same tooth type groups, being closer to the EDJ in preterm children and moving across its usual location at full-term birth towards the enamel surface during post-term gestation (Skinner & Dupras, 1993). The NNL separates a more regular microstructure in prenatal enamel from a less homogeneously structured postnatal enamel (Hillson, 2014). Thus, it can be considered as a point zero in chronological enamel development.

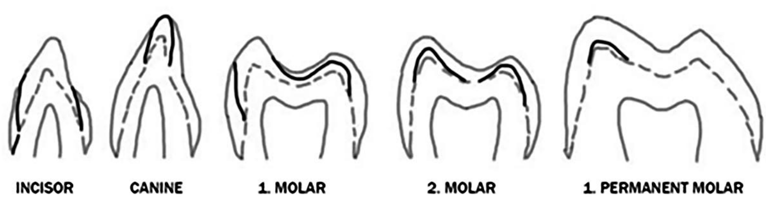

Figure 2. Location of the neonatal line. The four teeth on the left are deciduous (own drawing).

2.2 Enamel development in deciduous teeth

2.2.1 Amelogenesis

The enamel-forming cells, the ameloblasts, are large, column shaped epithelial cells, differentiated from the inner enamel epithelium first into pre-ameloblasts and eventually into ameloblasts, the differentiation occurs in communication with the odontoblasts. At the basal end of the cell which faces the developing enamel, is located a secretory organ called Tomes' process. The ameloblast's Golgi apparatus forms secretory granules, which migrate through Tomes' process to be secreted into the extracellular space. The secretion occurs from two sites of the Tomes' process: the intraprismatic enamel matrix is secreted from the distal end and the interprismatic from the proximal end. The first layers of enamel matrix are laid right next to the EDJ. Thus, ameloblasts withdraw or move from the EDJ towards the future enamel surface while the enamel matrix is formed behind them (Boyde, 1989).

One ameloblast produces one prism, a bundle of crystals. Approximately 1000 crystals are packed inside one prism, which is 3-7 µm in diameter (Robinson et al., 1995). Prisms run from the EDJ to the enamel surface. However, thin areas of enamel immediately after the EDJ and on the enamel surface are prism-free (Hillson, 2002). It has been shown that the amount of ameloblasts that start the formation of prisms from the EDJ is the amount that end up at the enamel surface (Risnes, 1998). When radial ground sections, usually 40-100 µm in thickness, are investigated in transmitted light microscopy with polarisation, at best prisms run parallel from the EDJ towards the enamel surface. The prisms are separated by a mineralised interprismatic space (Gustafson & Gustafson, 1967). However, the prism path is not straight: it undulates and may cut its way out the plane of sectioning. The prism layers that have different orientations deviating from parallel and showing rough unstructured zones, are called Hunter-Schreger bands, and appear as alternating brighter and darker bands. (Hillson, 2002) The undulations appear in the inner two-thirds of the enamel and, when strongest in the cuspal area, the prismatic structure is obscured, and the zones are called gnarled enamel (Hillson, 2014). Each prism is coated by a prism sheath, which contains organic material (Gustafson & Gustafson, 1967).

2.2.1.1 Neonatal line formation during amelogenesis

Prenatal enamel is relatively homogenous without interruptions because the uterus offers a protected environment for cellular function. Thus, most often the first disruption in enamel is the NNL caused by birth.

Amelogenesis is disturbed at the NNL, and this causes an optical phenomenon in microscopic views (Sabel et al., 2008). In transmitted light microscopy, the line is seen as darkened prisms, a sharpish band compared to the surrounding enamel, when the plane of sectioning shows parallel running enamel prisms (Weber & Eisenmann, 1971; Kodaka, 1996). In oblique cutting, the band is much wider and its lateral borders are diffuse (Weber & Eisenmann, 1971). Presumably, the NNL represents the degree of disruption. The section thickness, as well as the three-dimensional course of the NNL, also affect its appearance in light microscopy. A thicker section and a more oblique course of the NNL through the section lead to a greater amount of light scattering, enhancing the distinctiveness of the NNL but also obscuring structural details. (Hillson 2014). Weber and Eisenmann (1971) investigated the same tooth sections by progressively reducing their thickness. In approximately 150 µm thick sections the appearance of the NNL was blurred and broad, while in sections between 4 and 20 µm thick the region became lighter and narrower.

2.2.2 Enamel structure

The enamel matrix secreted by the ameloblasts has an organic component comprised of proteins, which control hydroxyapatite crystal growth and orientation. An inorganic portion contains apatite crystallites, kind of a grain of crystal, generating a framework for calcium hydroxyapatite formation (Hillson, 2002). The inception of the change from the enamel matrix into the fully mineralised form commences immediately after the secretion phase, maximally in tens of minutes (Boyde, 1989). Mineralisation is dependent on the blood flow to the enamel organ, which begins as enamel mineralisation begins continuing throughout maturation. (Boyde, 1989). The mineralisation of the enamel matrix involves a biological form of calcium hydroxyapatite formation, where the base unit is a hexagon-shaped hydroxyl column (Fig.3). The outer calcium ring in a hydroxyl column is shared with three adjacent hexagons and the formed 3-dimensional lattice is calcium hydroxyapatite, (Ca10(PO4)6OH2). These are laid on top of each other, forming a calcium hydroxyapatite crystal. Crystals can extend from the EDJ all the way to the enamel surface and are 50-100 nm in diameter. (Robinson et al., 1995)

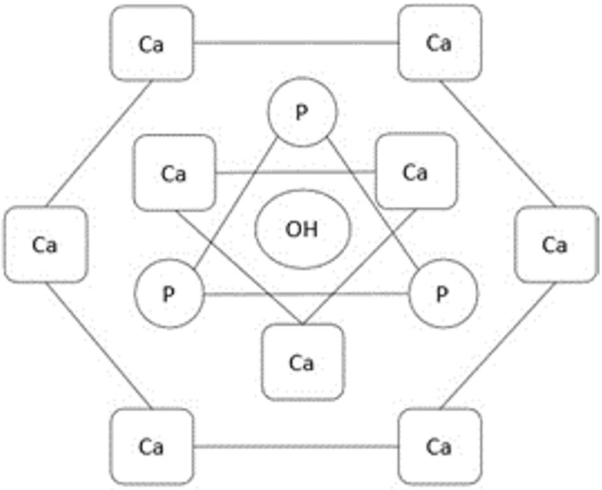

Figure 3. Hexagon-shaped hydroxyl column (own drawing).

When matured, enamel tissue is 95-97% mineralised in weight and 80-95% in volume. The major inorganic components are calcium and phosphate. Water may comprise up to 20% of the organic volume and approximately 2% of the weight. Other minor elements in enamel include carbonate, magnesium, fluoride and in very small amounts lead, zinc, strontium, and aluminium (Robinson et al., 1995). Mature, biological enamel still contains a tiny amount of enamel matrix proteins (Hillson, 2002).

2.2.2.1 Neonatal line structures

Microradiograph analyses have shown hypomineralisation at the NNL (Weber & Eisenmann, 1971; Kodaka, 1996; Sabel et al., 2008). Lower mineral content has also been detected in polarised light microscopy where the NNL appeared as a positively birefringent line and showed distinctively lower values of natrium and magnesium (Sabel et al., 2008).

Weber et al. (1971) as well as Sabel et al. (2008) reported the NNL to exhibit the staircase (Sc) configuration throughout its course. With a scanning electron microscope, Whittaker and Richards (1978) etched 200 ground sections of 300 µm thick, identifying the same Sc configuration in some of those, describing the phenomenon that the prisms running through the NNL changed direction in a zig-zag manner. They stated that the prism orientation after this interruption recovered to the same as in the prenatal enamel. Whittaker and Richards (1978) additionally reported a strong structural transverse change, about 0.2 µm wide, most likely expressing the ‘step’ of the Sc configuration. The Sc configuration was illustrated by Risnes (1990) with a three-dimensional model: decreased intraprismatic horizontal steps connected by increased interprism as vertical steps. Investigations of the NLL's and normal brown striae of Retzius' (bsR) Sc configuration showed that the horizontal step was introduced in two forms: an accentuated or crested prism expressing a lighter form of disruption and a prism cleft, a total transient pause in cell function (Wilson & Shroff, 1970; Weber, Eisenmann, & Glick, 1974; Whittaker & Richards, 1978; Risnes, 1990; Gwinnet, 1996).

2.2.3 Incremental formation of enamel

In normally developing enamel, the prism, throughout its length, is divided rhythmically into separate pillow-like squares. This pattern is thought to originate from a circadian function of the ameloblast. A 24-hour rhythm in enamel secretion has been reported by several studies (Risnes 1986; Boyde 1989; Dean, 1987, 1989), including studies of laboratory animals with injected markers (Bromage, 1991; Smith et al., 2006). Transverse lines, perpendicular to the long axis of the prisms causing the separate squares, are detected in ground sections for example under a polarised light microscope. The ameloblast has a slow and a fast phase in its secretion. The fast phase causes a swelling in the intraprismatic substance, whilst during the slow phase, the interprismatic substance causes a constriction inside the prism (Boyde, 1989). This phenomenon occurs simultaneously in all the ameloblasts along the enamel-forming front. Thus, enamel is formed in a circadian rhythm, and because of the periodicity of the detectable transverse lines, called prism cross-striations or short-period lines, the incrementally laid layers are countable (Fig. 4). Each daily increment is about 3-4 μm in length (Shellis, 1984; Risnes, 1998). Due to the circadian growth nature of the enamel, it is possible to count the past time in days both pre- and postnatally to any wanted enamel layer in relation to the NNL. This has been the basis for the gathered information on crown formation times (CFT) in deciduous teeth (Mahoney, 2011, 2012; Birch & Dean, 2014).

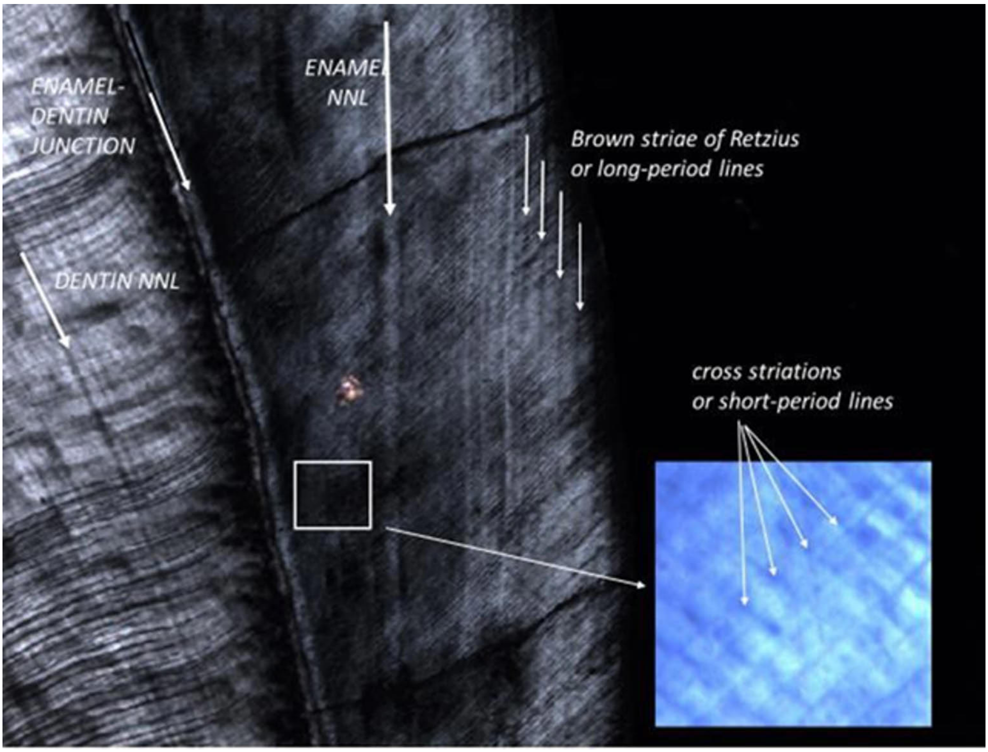

Figure 4. Enamel microstructures in transmitted light microscope with polarisation, magnification x 20. In high-power view of magnification x 40, the separate image of cross-striations is provided. A sample from the FDT collection.

2.2.3.1 Neonatal line increments and width

The possibility to count the incremental layers also applies along the NNL and the countable layers form the NNL width. The width of the NNL varies from only a few micrometres to even 30 µm and can be converted into days by dividing the length by four, the approximate daily increment length (Weber & Eisenmann, 1971; Eli et al., 1989; Kodaka et al., 1996; Sabel et al., 2008; Zanolli et al., 2011; Canturk et al., 2014; Kurek et al., 2015; Kurek et al., 2016; Hasset et al., 2020).

Schour (1936) stated that the NNL is caused by the metabolic changes resulting from the experience that the infant undergoes at birth and during neonatal life. Nóren (1983) claimed that this metabolic change is a decrease in plasma calcium (hypocalcaemia) which occurs in the first 48 hours after birth "since all infants show a decrease in plasma calcium shortly after birth, it is reasonable to assume that the neonatal line is a structural response to a stressed neonatal situation of hypocalcemia". Not enough knowledge yet exists on where exactly the actual birth event is located chronologically along the disturbed incremental layers that the NNL expresses.

Several studies have investigated NNL width in relation to gestational, perinatal and early life variables (Table 1). There has been some concordance between the results. Two studies have reported correlations with the mother's gestational health and medication. Hasset et al. (2020) showed the NNL to be thicker if the mother had gestational hypertension. Kurek et al. (2015a) reported a narrowed NNL if the mother had antispasmodic medication. The same two studies correlated seasonal variables of the time of birth with NNL width with incoherent results (Table 1). Mountain et al. (2021) showed that the mother's lifetime depression and psychiatric problems thickened the NNL. Hasset et al. (2020) additionally had significant correlation between late-term infants and thinner NNL that was in accordance with Zanolli et al. (2011), who reported pre-term males with thicker NNLs. This was explained by the more mature the child, the less external stress is experienced during birth, and vice versa.

Eli et al. (1989) were the first to report the correlation between NNL width and the mode of delivery. The results showed the thinnest disruption (7.6 ± 1.5 µm) in amelogenesis in caesarean sections, where the infant does not need to go through the birth canal during parturition. In normal vaginal deliveries, the NNL widths were within average values (11.9 ± 4.8 µm). Eli et al. (1989) thus suggested a general 12 µm width for the NNL. The use of forceps or vacuum during delivery caused the thickest lines (18.6 ± 5.7 µm), which they interpreted as extreme stress produced for the infant. Canturk et al. (2014) partially reproduced the same correlation between the NNL and mode of birth. Other studies investigating these parameters against the NNL width did not result in such statistically significant linear correlations (Table 1.).

Mountain et al. (2021) investigated self-reported lifetime mental health and social support of mothers after birth. According to their study, depression and other psychiatric problems were correlated with thick NNLs. High post-partum social support was related with thin NNLs. Kurek et al. (2015b) used a Polish archaeological tooth collection in their research and reported that low age-at-death (< 4 years) suggesting a troublesome early childhood correlated with a wider NNL.

Table 1. Studies of neonatal line width.

Publication	Collection	No of deciduous teeth	Teeth inc/can/mol	Microscopy	No of mea-sures	Measure location	Results Association with the NNL width	No association with the NNL width
Eli et al. 1989	Modern	147	23/62/40	transmitted light	3	cerv, mid, cusp	caesarean-normal-operative -> NNL width grows accordingly	
Zanolli et al. 2011	Modern	100	95/4/1	polarized transmitted light	6	mid of NNL	pre-term birth males -> NNL width is thicker	mode of delivery
Canturk et al. 2014	Modern	48	48/0/0	SEM	3	cerv third	caesarean-normal -> NNL width grows accordingly	
Kurek et al. 2015	Modern	60	60/0/0	SEM-BSE	3	cerv, mid, cusp	antispasmic medication ->NNL width is thinner born in spring/summer -> NNL width is thinner	caesarean section vitamins, iron and diseases during pregnancy
Kurek et al. 2016	Neolithic, medieval, modern	57	57/0/0	transmitted light	3	cerv, mid, cusp	low age-at-death (<4 years) ->NNL width is thicker	
Hasset et al. 2020	Modern	71	0/71/0	polarized transmitted light	3	cerv, mid, cusp	obese mother -> NNL width is thinner mother with hypertension -> NNL width is thicker post term birth -> NNL is thinner born in autumn -> NNL width is thinner	mode of delivery duration of delivery
Mountain et al. 2021	Modern	70	0/70/0	polarized transmitted light	3	cerv, mid, cusp	mother´s lifetime depression, other psychiatric problems -> NNL width is thicker mother´s high social support -> NNL is thinner	
NNL=neonatal line, inc=incisive, can=caninus, mol=molar, SEM=scanning electron microscope, BSE=back scattered electron, cerv=cervical, mid=middle, cusp=cuspal								

The tooth collections used in these studies vary. Some are archaeological and some modern. The tooth types between studies were different but mainly incisors and canines. Eli et al. (1989), unlike other researchers, pooled different tooth types in their collection. Other inequalities between studies include their varying number and location of measurements, as well as different types of microscopical methods. These may partly explain the variation in study results.

2.2.4 Disturbed enamel increments

2.2.4.1 Regular brown striae of Retzius

Whenever enamel increments are classified as abnormal or disturbed, changes can be detected in the prism path and structure and/or in mineralisation, compared to unperturbed enamel. Every 6th to 10th cross-striation, most commonly the 7th, 8th or 9th (Dean et al., 1993a), is affected by an unknown systemic cause, generating a more exaggerated short-period line in the enamel-forming front. These lines are called the brown striae of Retzius (bsR), or long-period lines, and they appear in all teeth in normal enamel development (Fig. 4). This phenomenon is called a circaseptan rhythm. The lines are best seen in the outer areas of enamel and appear in a light microscope as darker bands crossing the prisms. The spacing of these striae is always the same in one individual's dentition: two striae separate a constant number of daily increments (Hillson, 2002). The microstructural disturbance in the bsR is a discontinuity in enamel structure, often in the form of a Sc patterning crossing the prisms (Weber & Eisenmann, 1974).

As the bsR hit the amelogenesis throughout development, they eventually end at the enamel surface on the lateral walls, but always starting from the EDJ. On the surface of the enamel, the periodicity of the bsR is seen as horizontally lined up grooves and the whole view is called perikymata.

2.2.4.2 Irregular accentuated lines

Irregular enamel increments are called accentuated lines (AL), Wilson bands or pathological striae of Retzius. ALs, besides the NNL, are caused by internal or external physiological stressors (Rose et al., 1977; Rose et al., 1978; Dirks et al., 2002; FitzGerald et al., 2006; Gamble et al., 2017; Lorentz et al., 2019; Kierdorf et al., 2020) (Fig. 5). The disturbance can be sharp, covering only one or few increments, or extend over tens of daily increments. An abrupt change in prism direction, that is a bending of the prism, is a regular feature and in narrower ALs may be the only distinctive character. The prism bending can also delineate the dentin side of the disruption in particular, whilst the enamel surface side border is less sharp. The prism itself may be narrowed as the interprismatic substance has spread, generating an atypical prism form. The disturbance in cell activity causes variation in mineralisation, both hyper- and hypomineralisation. (Gustafson & Gustafson, 1967; Rose, 1977; Rose, 1979). Criteria for AL identification were proposed by Goodman and Rose (1990) as a) continuous at least ¾ of enamel thickness, b) matching both sides of the crown, c) appearance with oblique illumination as a trough or ridge, representing the sharp deviation of prism boundaries.

The underlying cause for disturbed enamel is systematic and affects all the developing teeth at a given time, both in deciduous and permanent dentition. The location of each formed disturbance thus varies within the tooth crown enamel, depending on the development stage of each tooth type and the amount of formed enamel. Besides the microstructure, the irregular incidence differentiates these disturbances from the regularly formed bsR. The repetitive appearance of the disturbance throughout the developing dentition offers an opportunity to combine isolated teeth belonging to the same individual (Boyde, 1989).

By now, studies have suggested several defined stressors as determinants of ALs. According to Birch and Dean (2014), incidents like inoculations (steroid, hepatitis B immunisation, DPT (diphtheria, pertussis and tetanus), H influenza type B and polio) and gastro-intestinal problems, vomiting and eye problems cause ALs. Their study was conducted on two deciduous mandibular molars from twin babies with known early life events, born 2 months preterm by caesarean section. The exact dates of the known events enabled the matching of the increment counts in relation to the NNL. Another study by Teivens et al. (1996) presented diseases like cytomegalovirus infection, nonspecific inflammation in kidney and spleen, severe cold and otitis and antibiotic treatment to be causes of AL formation. These babies in the study by Teivens et al. all deceased under the age of 1.5 years of sudden infant death syndrome (n=11) or infections and accidents (n=8). Teivens et al. investigated lower right mandibular teeth and matched the ALs with known early life events but concluded that exact positioning was not possible. On a study investigating a zoo-born female gorilla, an eye injury, followed by surgical treatment and anaesthetized hospital visits, caused accurately matched ALs (Schwartz et al., 2005). All the researchers above reported additional ALs in their specimen than the ALs that coincided with the recorded early life events, and also known life events which they were unable to match with ALs.

Skinner and Anderson (1990) used ALs to help in human identification. They investigated an unknown skull found in 1979 near the Taseko River, British Columbia, Canada, with no information to assess identity. Dental age assessment and anthropological study of the cranium indicated a five- to six-year-old boy, a putative match with a native boy reported missing in the area. Only in 1990 did these researchers manage to receive comprehensive health records for this alleged individual. They made a series of ground sections to cover a longer enamel development time, matched the repetitive ALs in subsequent teeth and were able to cover the time period from birth to five to six years. They corresponded ALs very closely to life events like surgery of soft cleft palate, food poisoning and asthma, thus confirming the identification.

In population-based tooth collection-studies of ALs, the researchers interpret ALs based on the published place- and era-specific context of the same population. Several studies have used the archaeological tooth collection from inhabitants of Portus Romae, Italy, from the 1st to the 3rd centuries AD. In terms of ALs, FitzGerald et al. (2005) investigated factors affecting AL formation at the time of stress. They argued "that the extent of the prismatic disruption seen in a Wilson band cannot be easily used to rank the severity of the stress event, since this may have as much to do with the timing and location in the crown and the tooth type than with the degree of physiological perturbation arising from the stressor". When the stressor hits the circaseptan rhythm coinciding with the timing of a bsR, a distinctive trace is formed. Between the Retzius lines, a more severe stressor is needed for an AL to be identifiable. In further research by FitzGerald et al. (2006), a topographical aspect of AL was presented. They suggested that ameloblasts in the middle third area of the tooth crown height are more susceptible to external disturbance. This was later supported by Nava et al. (2019), presenting a stress prevalence profile using both deciduous and permanent teeth from the same tooth collection as FitzGerald et al. (2006). In addition, FitzGerald et al. (2006) and Nava et al. (2019) reported a high AL prevalence peak which correlated with dietary transition when breast-feeding of a child transferred to consumption of solid food.

In medieval collections from Poland and Denmark, childhood stress was interpreted as high prevalence of ALs and was related to low age-at-death and gender- related age-at-death, respectively (Żądzińska et al., 2015; Gamble et al., 2017). Findings from Kierdorf et al. (2020) suggested that high levels of pre- and postnatal ALs expressed impaired maternal and infant living conditions in Shubayqa infants from a Late Epipaleolithic site in Jordan. A recent study by Kurek et al. (2020) indicated autism to increase individual's sensitivity to external stress, expressed by a higher number of ALs compared to a non-autistic control group.

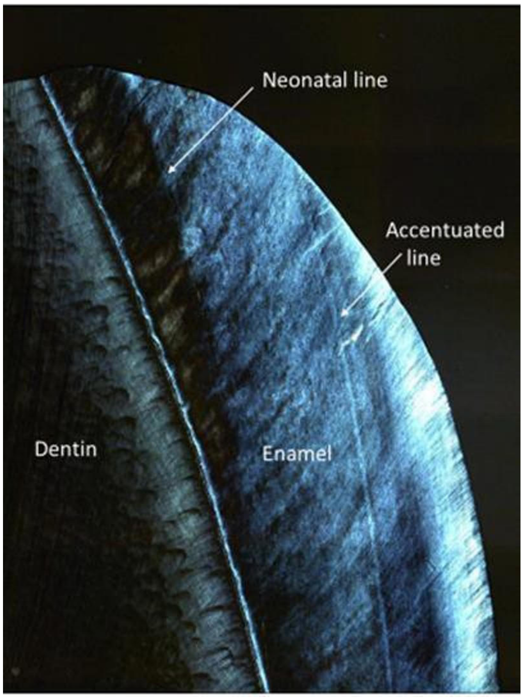

Figure 5. Polarised transmitted light microscope image, magnification x 20, showing a clearly visible accentuated line. A sample from the FDT collection.

2.3 Ground sections and microscopic methods

In routine laboratories, hard tissues such as teeth are softened by decalcification for sample preparation. However, this is not a suitable method for enamel studies since decalcification removes enamel from the tissue.

Tooth ground sections are very thin slices cut from the middle of the tooth crown. They are most often 40-100 µm thick. Viewing ground sections with microscope is the most common way to investigate microstructural features inside dental tissues. Processing a ground section requires special skills and experience in the laboratory. The sections need an ideal plane of sectioning, an even thickness throughout the sample and a highly polished, scratchless specimen. Researchers have preferences in materials and section thickness but follow the same overall process of preparing tooth ground section: cleaning, embedding, cutting, grinding and polishing. The method used is largely up to the researcher to evaluate, depending on the age and condition of teeth in use and the laboratory experience.

Cleaning removes fatty material and water. Cleaning the tooth can simply occur in 70% alcohol solution for 24 hours (Kurek et al., 2020). Hillson (2002) cleaned their teeth with a reflux of 50:50 chloroform:methanol in Soxhlet apparatus over two weeks continuously. For archaeological samples the recommended methodology is to immerse in 100% industrial methylated spirit (IMS, mixture of a larger portion of ethanol and smaller portion of methanol) for one day and then in a mixture of 1:1 IMS:chloroform for up to seven days (Hillson, 2002).

Embedding supports and strengthens the material. It ensures that cutting and grinding do not form artefacts in a specimen, as soft and hard tissues are worn differently. Embedding the sample involves immersing it in epoxy/polyester resin or polymethylmethacrylate (PMMA). At the simplest, the sample is placed in a small cup which is then filled with running epoxy or polyester resin and set to harden for up to two weeks (Dirks, 2002; Witzel, 2008; Kurek, 2020).

Cutting is executed by a hard tissue microtome with a diamond blade. Cutting produces a rough slice which is thicker than the aimed section thickness. It is crucial that the cutting creates a prerequisite for achieving an ideal plane of the final ground section: in incisors and canines strictly radial down the central axis, through the most incisal tip and straight buccal or labio/lingual or palatal direction. In molars, the cut is made most often through the mesial cusps and their underlying dentin horns. The final plane of section is evaluated by eye and can be marked with a pen on the resin block. The two cuts to engender the slice are eventually made on both sides of this marked section plane. (Hillson, 2002) Sectioning may also be performed horizontally to achieve a perpendicular view against the axis.

Grinding and polishing can be performed either manually or with a motorised tool, on both sides of the section. After the first microtome cut, this sample surface is ground and polished and fixed onto the glass slide. Then follows the second cut and grinding and polishing of the other side of the section. Grinding occurs with abrasive papers, starting with the coarsest (600) and progressively moving to the finer (up to 4000). Each grinding stage should remove the scratches from the previous stage. The scratches can be detected with a light microscope, holding the glass slide obliquely under the light. (Hillson, 2002). Final polishing is done with a motorised tool with diamond paste, which is spread on a rotating plastic mat, progressing from the roughest 3 μm grit to the finer, 1 μm and 0.25 μm. (Hillson 2002)

A transmitted light microscope is traditionally used to investigate ground tooth sections. The source of light is below the section and the view is bright with light brown structural features (Fig. 6 a). A polarising light microscope (POLMI) brings out more microstructural details and changes in mineralisation. The inorganic and organic components in a ground tooth section react differently to the light beam, a phenomenon called birefringence. The light is separated into two beams by one of the filters in the microscope, the polariser. The second filter, the analyser, constrains the beams into one plane and a scale in colour is created (Fig. 6 b). The colour depends on the section thickness, the birefringence property and the orientation of

the inorganic and organic components. Grey and yellow shades are created with 100 µm thick sections. (Hillson, 2014)
A scanning electron microscope (SEM) can convert the magnification into even x 200 000. With an SEM, a beam of electrons scans the surface of the specimen, and the image is formed gradually on the screen attached to the microscope. A SEM is used with two detector types, back-scattered electron (BSE) and secondary electron (SE). With BSE-SEM, the beamed electrons mostly stay within the specimen, but those that escape and scatter react in varying rates to the degree of the mineralisation and the topography of the specimen. A BSE-SEM produced image has hues of grey to express the degree of mineralisation and the surface as a two-dimensional relief (Fig. 6 c). More mineralised areas are seen as brighter than less mineralised areas. Electrons hitting the specimen may initiate secondary electrons, SE-SEM, signalling topographical changes. (Hillson, 2002, 2014)

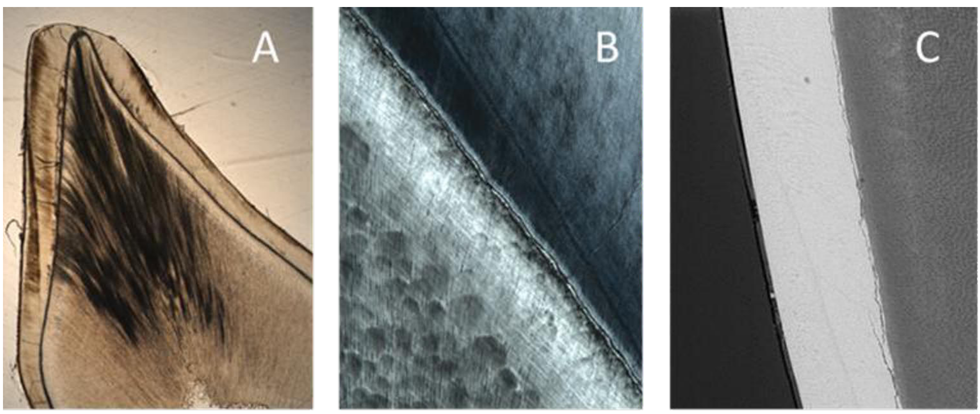

Figure 6. Images of a ground tooth section with a) a transmitted light microscope, objective x 2,5 and b) a polarised light microscope, objective x 20 and c) a back-scattered scanning electron microscope (BSE-SEM) magnification x 200. Samples from the FDT collection.

3 Aims

Life events disturbing ameloblast function have not been comprehensively studied. With our collection of modern deciduous teeth with known gestational, perinatal and early life background data, the aim was to further investigate factors affecting the neonatal line width and structure and the occurrence of postnatal enamel accentuated lines.

The specific aims for each study were:

1. To analyse the effect of duration and mode of delivery on the neonatal line width.
2. To document the presence of a staircase -pattern in the neonatal line in different tooth types and to investigate its relation to mode and duration of birth and use of pain-relieving medications.
3. To investigate the occurrence and location of postnatal accentuated lines in the tooth crown enamel, and to evaluate the associations between early life events and the occurrence of accentuated lines.

4 Materials and Methods

4.1 Materials

4.1.1 Finnish Family Competence study

The Finnish Family Competence (FFC) study is a longitudinal population-based follow-up study launched in January 1985. The study design was approved by the Joint Ethical Review Committee of the University of Turku and Turku University Hospital (DNO 540/582/85). The main goal of the FFC study was to survey "the way of life and health behaviour of young Finnish families in order to develop and improve the existing public health services in the fields of health education and socio-emotional support and to clarify the development of the child's health and way of life" (Rautava & Sillanpää, 1989).

The source population in the FFC study originated from two central hospital regions situated in South-western Finland (Turku University Hospital Region, Turku, Finland, and Satakunta Central Hospital Region, Pori, Finland) which were responsible for catchment areas with a joint population of 713,050 inhabitants at the end of 1985. Subject collection was based on stratified randomised cluster sampling. Of 1,713 young families expecting their first child, 1,443 signed consent to participation. The families were able to cease participation in the study whenever they wanted. The data collection started on 1 January 1986. Mothers were recruited in about the 10th week of pregnancy at their first visit to the maternity health care clinic. Sociodemographic, socioeconomic and medical data for the families and their children were prospectively collected. The questionnaires were given at the 10th and 28th weeks of pregnancy, at birth and further on six visits to well-baby clinics until the age of seven years. The information collected covered the time period between each visit. The questionnaires were addressed to mothers, fathers and health care professionals at each visit time. Socioeconomic status was defined according to the criteria of the Central Statistical Office of Finland based on the United Nations recommendations (Central Statistical Office of Finland, 1989; (Rautava & Sillanpää, 1989). The study design and follow-up of the cohort has been described previously (Paunio et al., 1993; Mattila et al., 2001; Sourander et al., 2006; Sillanpää et al.,2008; Jaakkola et al., 2013).

4.1.2 Finnish Deciduous Tooth collection

Teeth

During the ongoing FFC study, of the 1,285 participants, 358 could still be located in Turku by the time the mothers were requested to deliver the loosened deciduous teeth of their child in question (Mattila et al., 2001). One tooth from each of 146 children was received from late 1995 to the beginning of 1997. A form attached to the tooth at collection stage categorised it as maxillary/mandibular and left/right. Nine teeth were broken and in seven teeth the labelling was not clear enough to attach them to the correct individuals. Thus, 129 deciduous teeth form the tooth collection called the Finnish Deciduous Tooth (FDT) collection.

The collected teeth were transported dry to the researchers and thereafter preserved in neutral formalin as soon as possible. The teeth were processed in the histology laboratory of the Institute of Dentistry, University of Turku, Turku, Finland by a skilled laboratory technician trained in hard tissue methods, following a protocol established at the laboratory and according to the material and machine manufacturers' instructions. To start the ground section process, the samples were half-cut in the bucco-lingual/palatal and axial direction, through incisal middle or cusp tip and underlying dentin horn using an EXAKT 300 CP Band System and (EXAKT Technologies, Inc., Oklahoma City, USA). The plane of section was estimated free-handed. One half of each sample was preserved in neutral formalin and one half was processed further. The samples were then run through a rising alcohol series (70%, 96%, 100%), at each stage for two to three days at room temperature, to remove impurities. Next, they went through resin and alcohol treatment (Technovit/alcohol (50%/50%)) for two to three days, and two pure resin treatments, first for two days and then for one to two days, to strengthen the tooth structure (Technovit; Heraeus Kulzer, Hanau, Germany). The samples were then embedded in resin (Technovit 7200) and hardened into blocks with three hours in plain light followed by ten hours in blue light (EXAKT 520 Light Polymerization Unit, EXAKT Technologies, Inc., Oklahoma City, USA). Preliminary moulding (cutting the excessive outlining block parts) and grinding with 800 and 1200 abrasive papers (EXAKT 400 CS Micro Grinding System, EXAKT Technologies, Inc., Oklahoma City, USA) was done to make the block thickness even. In each sample, the back of the resin block was glued with cyanoacrylate adhesive, Loctite (Henkel, Düsseldorf, Germany) onto a glass slide. The exposed sample surface was to be the back of the final ground section. Its surface was ground with 800 and 1200 abrasive papers to just slightly aside from the ideal plane of section and then glued with Technovite 7200 VLC to a glass slide. From the engendered `sandwich' a slice was cut towards the latest glued glass slide and the newly exposed surface was ground with 800 and 1200 abrasive papers to the point of the ideal section plane. The final product was a ground section of a 20 µm thickness.

In the three studies of this dissertation research, the material, meaning the number of teeth, were selected differently depending on the aims of the study and thus, the preservation of the features to be investigated in the tooth crown enamel (Table 2 and Table 3).

Table 2. Number and criteria of deciduous teeth in the three studies.

Study	Aim	Requirements for chosen samples	Total sample no
I	Neonatal line identification in the collection	All available teeth	129
	Neonatal line width and the effect of delivery on it	Group A: Teeth with no to moderate enamel wear with a sharp dentin horn to reassure the most ideal plane of sectioning achieving the most genuine width of the neonatal line	54/129
	Not included in the neonatal line width analyses	Group B: Teeth with heavier wear to dentin, which does not guarantee an ideal plane of sectioning	75/129
II	Presence of staircase in the whole neonatal line and its relation to known intrapartum events	Teeth with complete and clear neonatal lines in both crown walls	88/129
III	Prevalence of accentuated lines, location within enamel, and associations with early life events	Teeth with well-preserved crown showing accentuated lines along whole tooth crown	61/129
No = number			

Table 3. Number and type of deciduous teeth in the three studies.

Study	Total	Incisive	Canine	Molar		Boy/girl
I	129* A:54 B:75	12 3 9	62 20 42	55 31 24		57/72 21/33 36/39
				1. molar	2. molar	
II	88	3	58	22	5	
III	61	3	40	13	5	
*only one tooth was received from each child						

Life events

The background data covered the time period of deciduous teeth enamel formation. The included life events shown in Table 4 were collected from questionnaires to the mothers and health care professionals covering the time period from the beginning of birth until 18 months, including data recorded from the questionnaires at the 10th and 28th weeks, birth, 3, 9 and 18 months.

Table 4. Life events compared to accentuated line formation of deciduous teeth.

Mother	
Pregnancy	Diseases, allergies, traumas, medications and other treatments Operations Urine proteins/glucose, severe contraction before delivery, hypo/hyperthyroidism
Mother/Child	
Birth	Gestation weeks, mother's pain relief medication, mother's contraction-enhancing medication, mode and duration of delivery
Perinatal-care	Child: care in neonatal intensive unit and in ward, age in hours when perinatal care started, indication, duration of perinatal care Mother: breastfeeding, puerperium infections and medications
Child	
0–18 months	Abnormalities, disabilities and their treatments Diseases, allergies, traumas, medications and other treatments Operations Vaccinations

Before the age of one year the Finnish vaccination programme in the 1980s comprised of BCG (tuberculosis) within the first week (0-7 days) of life, then DTP (diphtheria, tetanus, whooping cough) at three, four and five months and IPV (polio) at six months. All the mothers stated they had followed the programme.

4.2 Methods

4.2.1 Method of neonatal line width measures (Study I)

All samples (n=129) were investigated by one author (JH) with a transmitted light microscope to identify the NNL (Leica DM600B attached to Leica DMC2900 camera, Solms, Germany) with objective lenses 1.25x/0.04, 2.5x/0.07, 5x/0.15, 10x/0.40 and 20x/0.70. The most middle third of the crown enamel (visually, not accurately measured) on the buccal or labial crown wall was focused on with a x 10 objective lens and a photograph was taken. The NNL width was measured with a digital ruler. Six separate measurements were taken along the prism path in the area of focus. Measurements were independently repeated by another investigator (VV) without knowledge of the previous findings in 44 randomly selected teeth to evaluate inter-observer variation.

4.2.2 Method to record the staircase-pattern neonatal line (Study II)

The 88 samples with total and clear NNLs in both crown walls were investigated with linear polarised transmitted light using a Leica DM6000B microscope (Leica, Solms, Germany) with 10x, 20x and 40x objective lenses to record the absence and presence of an Sc pattern (Sc0 and Sc1 respectively) along the NNL. Sc configuration is expressed as a decreased intraprismatic horizontal step connected by increased interprism as a vertical step. Digital micrographs were obtained with a Leica DMC2900 camera (2048x1536 pixels). The NNL in the buccal/labial and lingual/palatal walls were divided into halves of its length, lower and upper parts, and Sc0 (absent) and Sc1 (present) in both crown walls were recorded separately in both parts. The character was qualified only when both horizontal and vertical steps were visible clearly enough to create a Sc configuration and was dominant in each part. In addition, strong/crested cross-striations were recorded, where an Sc-related vertical step was missing.

All 88 samples were analysed by one observer (JH) with x10 and x20 objective lenses the first time and re-evaluated after a month or more for intra-observation reasons. Next, another observer (CW) evaluated eleven randomly selected samples to validate the Sc configuration.

Absence (Sc0) or presence (Sc1) and crested cross-striations in the NNL was systematically listed in relation to tooth type (incisor, canine, 1. molar and 2. molar) in both crown walls and compared to the perinatal medical data: mode of delivery (spontaneous vaginal, assisted vaginal or caesarean section), duration of delivery and use of pain-relieving medication (nitrous oxidative, epidural anaesthesia, paracervical injection).

4.2.3 Method to record accentuated lines (Study III)

Tile scan images of 61 samples with well-preserved crown showing postnatal ALs along whole deciduous tooth crown were processed with a x 10 objective lens and 1.6 magnification using a polarised transmitted light microscope, Leica DM6000B, and its motorised table to cover images of the whole buccal or lingual crown enamel. Digital data for the tile scans were obtained with a Leica DMC2900 camera (2048×1536 pixels). The data of ALs from the tile scans were gathered later using Leica LAS X software.

The ALs were identified in blinded fashion without knowledge of the background data. One researcher (JH) analysed all the samples twice with at least one month in between. The locations of ALs were measured using the following method: the starting point was where the NNL crosses the EDJ. The first measurement was taken from this point along the prism path to the first identified

AL. This AL was then followed cervically to the point it crosses the EDJ, where a new measurement was initiated to the second recorded AL. This procedure was repeated to the last identified AL. The gained measurements were converted into days using tooth type-specific regression formulas presented by Birch and Dean (2014). Thus, by adding the calculated daily amounts, all the ALs in the crown enamel could be approximately dated.

Ear infections and skin diseases occurred within 0-90 and 91-270 days; in vaccinations it was presumed that vaccination was given within the first week, then between 75-105 days, 106-135 days, 136-165 days and 166-195 days according to the 1980s vaccination programme at (0-7 days of life BCG (tuberculosis), three, four and five months DTP (diphtheria, tetanus, pertussis (whooping cough) and six months IPV (polio)).

By the time of the request for the loosened deciduous teeth, there were 1,285 participants involved in the FFC study. In the dropout analysis between the 129 participants who delivered a tooth for the FDT collection and the 1,156 participants with no teeth available for analysis, socioeconomic and sociodemographic parameters showed significant differences only for a higher mean age (+1.5 years) of the mothers at the time of delivery ($p<0.001$, t-test) and their residence, which was mostly in an urban area ($p<0.001$, Fisher's Exact Test). The socioeconomic status or boy/girl distribution of children in the FDT group were not significantly different from the general population ($p=0.14$).

4.2.4 Statistical methods

One-way analysis of variance in all samples (n=129) was used to compare the NNL widths between the sexes. In 54 well-preserved samples (Group A: Teeth with no to moderate enamel wear), one-way analysis was used to show differences of width between delivery modes (caesarean section, spontaneous vaginal and assisted vaginal delivery). Logarithmic transformation was used on the NNL width to achieve the normality assumption. The association between the NNL width (log transformed) and duration of delivery was calculated using the Pearson correlation coefficient, and the inter-observer variation between two observers was also evaluated. Teeth in group B, with heavier wear to dentin, were left out from the previous analyses, because wear may disturb achieving the ideal plane of sectioning and thus bias the NNL measures (Study I).

The prevalence of the Sc NNL was first calculated between all tooth types with Fisher's exact test and extended with an additional Chi-square test for pairwise comparison of tooth types. The canines and separate crown walls of the deciduous molars were set in line according to the growing ratio of prenatal enamel in relation to total crown enamel (data provided by Mahoney (2011, 2012) and Birch and Dean

(2014)). The Cochran-Armitage trend test analysed the increase of Sc1 along the line. The effect of the mode of delivery and use of pain medication on Sc prevalence was examined using Fisher's exact test and for the not normally distributed duration of delivery measurement data with the Wilcoxon rank sum test (Study II).

Vaccinations in all samples (n=61), acute suppurative otitis media with drum rupture combined with antibiotics (n=15) and erythematous conditions and other atopic dermatitis and related conditions (n=6) appeared most often in the FDT background data. Whether these events were likely to result in an AL was evaluated with the proportion of having an AL present within the same time period in which an event was recorded, and the 95% confidence intervals were calculated for the proportions. Due to the coinciding life events, with vaccinations statistical analyses were conducted two ways: the assumptions that vaccination 1) when coinciding with another diagnosis, did not cause an AL and that vaccination 2) when coinciding with another diagnosis, did cause an AL. Without the knowledge of exact dates, in this way we tested the minimum and maximum effect of a vaccination in causing an AL (Study III).

P-values of less than 0.05 (two-tailed) were considered statistically significant. The analyses were performed using the SAS System, version 9.3 for Windows (SAS Institute Inc., Cary, NC, USA) and JMP® Pro, Version 13.1.0.

5 Results

Within the FDT collection (n = 129) the sociodemographic and socioeconomic background analysis delineated the mean age of the mothers at delivery being 27 years (SD 4.36, median 27, range 17–38 years). One hundred and twenty-one mothers lived in urban areas and 8 in rural areas.

Of the mothers, 105 had no long-term (>6 months) diseases. One mother was diagnosed with hypothyroidism and one with hyperthyroidism. Twenty-two mothers advised of un-specified allergies. The most prevalent medical conditions of mothers during pregnancy were non-inflammatory disorders of the cervix, disorders of the urinary tract and diseases of the respiratory system.

All 129 children whose deciduous teeth were received followed the Finnish vaccination programme. Of these children, 26 (20%) had no life events recorded during their first year of life. The most prevalent diagnoses of the rest in the background data were common diseases of infants such as acute suppurative otitis media with and without antibiotics, acute laryngitis and nasopharyngitis in all time ranges (0-3, 3-9 and 9-18 months). In addition, eczema, atopic dermatitis and other atopic dermatitis were recorded for some individuals. Similarly, ventricular septum defect, secundum atrial septum defect and heart injury (not otherwise specified) were diagnosed as congenital disorders in individual children.

5.1 Neonatal line width and its correlations with mode and duration of delivery (Study I)

The NNL was identified in 126 out of 129 (98 %) of the deciduous teeth in the FDT collection. All tooth types (incisor, canine and molar) were represented in the three samples (2%) with no detectable NNL. The NNL width varied from 3.16 µm to 27.58 µm, the median width being 9.63 µm. The Pearson correlation coefficient in the inter-observer variation was 0.95. The NNL width did not differ between boys and girls (p=0.39).

The analyses for correlation between the NNL width and mode and duration of delivery were made for samples with no or moderate wear (group A, n=54) (Table 2). Duration of delivery was defined to start when contractions appeared at five-minute intervals and to finish when the child was born. Duration of delivery in vaginal deliveries (spontaneous n=28, assisted n=11) correlated significantly with the NNL width (in all vaginal deliveries r=-0.41, p=0.0097). The longer the duration of delivery, the narrower the NNL (Fig. 7). The mode of delivery did not correlate with the NNL width (p=0.36) (Fig. 8).

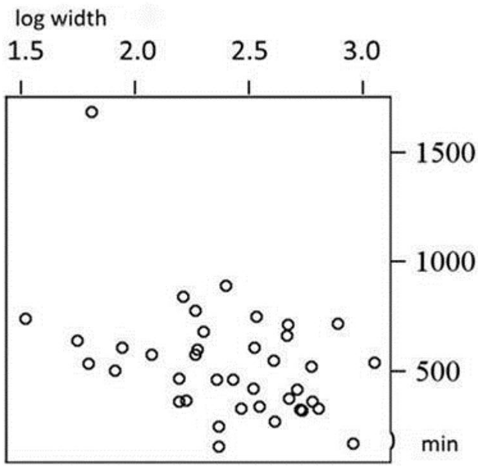

Figure 7. Pearson Correlation Coefficients (r=-0.41, p=0.0097) of neonatal line width (log) and duration of delivery (min) in vaginal deliveries (n=39).

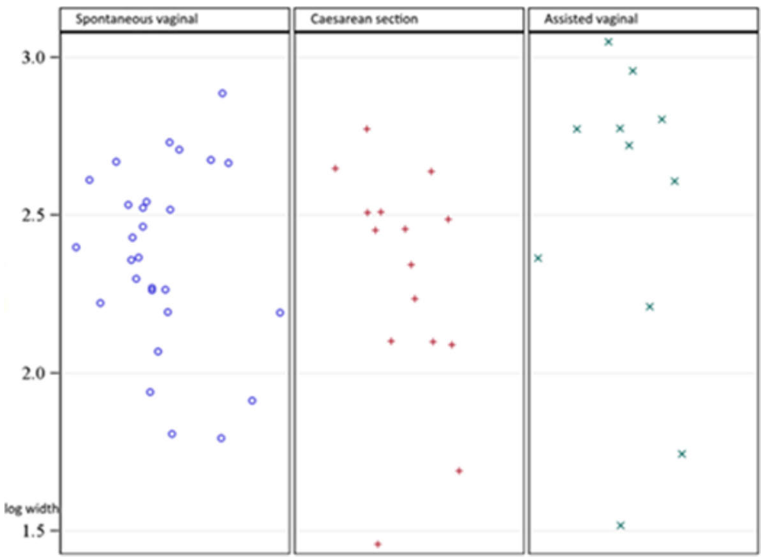

Figure 8. Differences of neonatal line width (logarithmic transformation) between delivery modes. One-way analysis of variance, p=0.36. Purple, red and green characters address logarithmic transformations of neonatal line widths.

5.2 Staircase-pattern neonatal line prevalence and its association with tooth type and perinatal events (Study II)

The Sc -pattern was absent (Sc0) in 68 (77%) samples and visible (Sc1) in 20 (23%) samples from the 88 teeth investigated. Observer variation evaluated with the Simple Kappa Coefficient was 0.73 for intra-observer and 0.84 for inter-observer.

The prevalence of Sc was different between different deciduous tooth types (3 incisors, 58 canines, 22 1. molars and 5 2. molars), statistical significance $p<0.0001$. When the analyses were made pairwise between different tooth types, the prevalence of Sc differed significantly between incisors and canines ($p=0.0027$), canines and 1. molars ($P<0.0001$) and 1. molars and 2. molars ($p=0.0005$), but no significant association was shown between incisors and 1. molars ($p= 0.065$), incisors and 2. molars ($p=0.17$) and canines and 2. molars ($p=0.77$). Deciduous teeth start to develop in turns. These results indicated that difference in Sc prevalence was dependent on the location of the enamel-forming front in the crown enamel height at the time of birth.

The prevalence of Sc was further analysed between separate crown walls. Crown walls were set in a sequence in which the proportion of prenatal enamel in relation to total crown enamel increased. The proportions were obtained from previous studies (Mahoney, 2011, 2012; Birch & Dean, 2014). The Cochran-Armitage trend test showed that Sc was more likely to be present when the amount of prenatal enamel increased ($p<0.0001$). Only one canine showed an Sc feature. The existence of Sc (Sc1) in first deciduous molars on the buccal/labial side was 50% (11/22) and the lingual/palatal side 55% (12/22) and Sc did not appear in second molars (n=5). Adding the scored strong cross-striations (canines n=1, first molars n=8 and second molars n=1) to the present recordings (strong cross-striations + Sc1) emphasised the rising Sc1 percentages along with the increasing amount of formed prenatal enamel. The results indicate that the more prenatal enamel is formed, the more likely an Sc pattern is formed. The prevalence of Sc was further analysed between separate crown walls (Table 5)

Table 5. Number and percentage of staircase NNL in lingual and buccal crown walls in tooth types according to the rising prenatal enamel amount.

prenatal enamel % of total crown enamel	Teeth	Staircase			
		absent	%	present	%
ling/bucc		ling/bucc		ling/bucc	
15%/19%	2.molar (5)	5/5	100%/100%	0/0	0%/0%
20%/20%	caninus (58)	58/57	100%/98%	0/1	0%/0.02%
29%/29%	1.molar (22)	10/11	45%/50%	12/11	55%/50%
Ling = lingual, bucc = buccal					

The delivery modes of the 88 cases were 51 spontaneous vaginal (58%), 20 assisted vaginal (23%) and 17 caesarean sections (19%). The mode of delivery was not associated with Sc existence in the NNL (p=0.65). Duration of delivery varied in vaginal deliveries from 2h 50min to 28h 8min (median 9 h) in 67 (76%) of 88 mothers, in 47 spontaneous and 20 assisted deliveries. Data on the delivery length were missing from the 17 caesarean sections and from four cases with spontaneous vaginal delivery. The duration of delivery did not affect the presence of Sc (Sc1) (p=0.57).

Altogether, 63 (72%) mothers used pain-relieving medication during their deliveries. The use of pain-relieving medication did not have an impact on the emergence of the Sc feature along the NNL (p=0.58).

5.3 Accentuated line occurrence in deciduous tooth crown postnatal enamel and the association between accentuated lines and early life events (Study III)

Altogether, 58 ALs in 37 teeth were identified in the deciduous teeth (n=61) (Table 6). The number of ALs in an individual tooth varied from none to five. ALs were most often found in canines. Thirty-six of a total of 48 ALs in canines (75%) appeared in the two middle quartiles, and 27 (56%) in the middle third. Intra-observer variation agreement in AL identification was evaluated with the Simple Kappa coefficient as 0.67.

Table 6. Number of teeth with/without accentuated lines according to tooth type and number of accentuated lines divided by tooth type.

	Total number	Incisors	Canines	1.molars	2.molars
All teeth	61	3	40	13	5
Teeth with accentuated lines	37	2	29	4	2
Teeth with no accentuated lines	24	1	11	9	3
Accentuated lines	58	2	48	4	4
N = number					

Vaccinations (n=61), acute suppurative otitis media with drum rupture combined with antibiotics (n=15) and erythematous conditions and other atopic dermatitis and related conditions (n=6) appeared most often in the FDT subsample background data. We evaluated the associations between these three early life events and the occurrence of ALs (Table 7). From the analysis of the vaccinations, three incisors were rejected, because their crown formation times do not reach all the vaccination times included in this study. In all vaccination programme time ranges we found an indication that vaccinations do not regularly engender ALs. Even with the largest amount of supposed vaccine-related ALs, as a rule, vaccination was not a strong enough stressor to result in AL formation. Similarly, ear infections were not likely to generate an AL (n=15). Skin diseases showed the highest probability, however in a small sample size.

Table 7. Proportions of ALs and 95% confidence level.

Finnish vaccination program	Proportion of ALs with assumption that vaccines are not determinants of ALs when coinciding with another event	Proportion of ALs with assumption that vaccines are determinants of ALs when coinciding with another event
0-7 days	no ALs	no ALs
3 kk	5.2% (95% CI 1.77% to 14.1%)	10.3% (95% CI 4.83% to 20.79%)
4 kk	15.5% (95% CI 8.38% to 26.9%)	22.4% (95% CI 13.59% to 34.66%)
5 kk	6.9% (95% CI 2.71% to 16.4%)	12.1% (95% CI 5.97% to 22.88%)
6 kk	10.3% (95% CI 4.83% to 20.79%)	17.2% (95% CI 9.64% to 28.91%)
Background data time period including ear infection recordings	Proportion of ALs with assumption that ear infections are determinants of accentuated lines	
90-270 days	26.7% CI [7.8%, 55.1%]	
Background data time period including skin disease recordings	Proportion of ALs with assumption that skin diseases are determinants of accentuated	
0–270 days	66.7% CI [22.2%, 95.7%]	

6 Discussion

6.1 NNL occurrence

The NNL was identified in 126 out of 129 samples (Study I). Our finding of NNL prevalence and varying width is supported by the literature (Eli et al., 1989; Zanolli et al., 2011; Canturk et al., 2014; Kurek et al., 2015, 2016; Hasset et al., 2020). The explanation behind the three samples in our study with unidentifiable NNL may be the use of a transmitted light microscope. A light microscope can be insufficient to show the faintest microstructural changes of the NNL. In addition, even though hypomineralisation is noticed along the NNL compared to the surrounding enamel (Weber & Eisenmann, 1971; Sabel et al., 2008), the original difference may be too minor, and the enamel may later mineralise, causing the NNL to become unnoticeable (Birch and Dean, 2014).

6.2 NNL and duration of delivery

Duration of delivery had an effect on NNL width in our study. The longer the duration of delivery, the thinner the NNL (Study I). We suggest that in prolonged deliveries, possibly intensified with the use of forceps or suction cup, the functioning of the ameloblast may transiently cease under stress and consequently narrow the NNL. This is supported by Witzel et al. (2008), who suggested that the progressively increasing external stress may cause impairments in ameloblast function. Microstructurally the ceased function is specified as a cut in the prism path, called a prism cleft, followed by an immediate continuance of the ameloblast function. Such structural details are best seen with higher magnifications with POLMI or especially with SEM. We used a transmitted light microscope in Study I and could not however differentiate such details at that point. The connection of the NNL width to the duration of delivery was later tested by Hasset et al. (2020), who were unable to show statistically significant results.

6.3 NNL and mode of delivery

Our results showed that the mode of delivery had no effect on NNL width. The tooth types and the use of a transmitted light microscope in our Study I were comparable to the study by Eli et al. (1989), who claimed a reasonable explanation for the varying NNL width. For 'normal' vaginal delivery they found an average NNL width of 12 µm. Caesarean section was considered as a quick and easy birth for child and therefore caused thinning of the NNL. Operative vaginal birth assisted with forceps or suction cup caused more stress for the child and showed the thickest NNL lines. However, compared to the study by Eli et al. (1989), we simplified the measurement methodology. Instead of executing the incisal, middle and gingival NNL width measurements, as Eli et al. (1989), our measures were completed in the most possible middle third of the crown enamel because the NNL is not yielded through the whole vertical crown enamel in all tooth types. We failed to reproduce Eli and his group's results and neither have other authors published such linear association between NNL and mode of delivery (Zanolli et al., 2011; Canturk et al., 2014; Kurek et al., 2015; Hasset et al., 2020).

6.4 Reasoning behind differing results of NNL width studies

The lack of consensus in the published NNL width studies may be explained by the variation in the used tooth type collections. We found that during the stress of birth, canine NNL does not seem to be prone to microstructural Sc formation, which could bias the forming NNL width (Study II). Therefore, we recommend canines to be the most appropriate tooth type for NNL width studies. In the FDT collection, we have a good number of canines, a tooth type also used by Hasset et al. (2020). Therefore, the association of NNL width with the mode and duration of delivery can be revisited. To date, rather than investigating perinatal variables, some coherent evidence exists to support that variables during mother's gestation have an impact on NNL width. Hasset et al. (2020) evidenced that mother's gestational high blood pressure caused thicker NNLs, whereas Kurek et al. (2015) found that medication for this same disease made NNLs thinner. In addition, Mountain et al. (2021) stated a growing width of the NNL as a result of the mother's psychiatric problems. Gestational registered variables are included in the FDT background data and can be additionally investigated.

6.5 Effect of the location of the forming enamel front under stress

Prism continuation, strong cross-striations and Sc configuration along the NNL varied in different areas of deciduous tooth enamel (Study II). The Sc view of the NNL was first reported in 1978 (Whittaker & Richards, 1978; Sabel et al., 2008). Our results evinced that Sc view in the NNL was associated with the NNL location within the crown enamel (Study II). Weber et al. (1971) showed that by reducing the ground section thickness from 20 to even 4 µm, the Sc pattern became more visible. However, the section thickness is not the only possible explanation, since we also showed strong Sc correlation with the deciduous tooth type in question in the FDT collection samples, which are all 20 µm thick ground sections. We showed that the more prenatal enamel had formed, expressing an older life span age of the ameloblast, the more the Sc pattern appeared in the NNL. Besides the effect of the formed prenatal enamel, a factor that seemed to have an even stronger effect was the vertical location of the line. The more the NNL formed in the middle third of the tooth crown enamel height, the stronger the differentiated ameloblasts responded to form a Sc pattern. This indicates that there is variation in the areas of enamel where ameloblasts along the enamel-forming front line are more susceptible to internal or external stress. Considering this, we also found the recorded disturbed enamel increments, ALs, to be most prevalent in the middle third of the tooth crown (Study III). This topographical ameloblast susceptibility is supported by FitzGerald and Saunders (2006). However, their interpretation included the caution that it is not certain whether this is due to a different visibility of microstructural changes in different crown areas.

We noticed that at the time of birth the ameloblasts in the first deciduous molars were more susceptible to birth stress, as the prevalence of the Sc pattern was highest along the NNL. The Sc pattern was dominant in first molar NNLs; however, there was a variation in appearance. Thus, we suggest that first deciduous molars, as a larger collection, might be an appropriate tooth type for investigating the NNL microstructural variability associated with various birth-related stress factors. In contrast, canines failed to show the Sc pattern (Study II). Canines were previously recommended to be used in NNL width studies.

The horizontal step of a Sc, described as a cleft, is considered as a pause in cell function. The forming prism is cut off and a new prism starts as an immediate continuance after the cleft and directionally the same as in the prenatal enamel (Wilson & Shroff, 1970; Weber, Eisenmann, & Glick, 1974; Whittaker & Richards, 1978; Risnes, 1990; Gwinnet, 1996). This pause was speculated to be the cause of thin NNLs as consequences of stressful vaginal deliveries (Study I). However, the use of POLMI to detect the Sc patterned NNL (Study II) showed that practically none were identified in the canines which were included in the samples in our first study. Thus, our previous reasoning in Study I, where the thin NNL was interpreted as a pause in the ameloblast function, causing a cleft in the prism, is not, as a rule, supported by our results of Study II.

6.6 Life events and ALs

ALs have been suggested to express experienced stress (Rose, 1977; Rose, Armelagos & Lallo, 1978; FitzGerald, Saunders, Bondioli, & Macchiarelli, 2006). Their irregularity differentiates ALs from the regularly appearing bsR which appears in undisturbed enamel. The NNL, as a point zero in enamel, and the knowledge of the daily incremental formation of enamel, offer an opportunity to explore whether an AL is an expression of a known and exactly dated life event (Schwartz et al., 2006; Birch & Dean, 2014). This evidence is quite strong, even though scarce. Medicine could benefit from future clinical studies proving ALs' connection to a disease and its time of occurrence.

Amongst the examined 61 teeth, 37 showed ALs. The low approximate appearance of 1.5 per sample in our modern collection compared to an archaeological sample of FitzGerald et al. (2006) most likely illustrates the better life conditions and advancement in medicine today. We had 24 samples with no ALs, related to and not related to previous history. On the other hand, 37 samples showed ALs, yet only in 20 did life events exist. Therefore, it is reasonable to conclude that the aetiology of other existing ALs than the NNL is unclear. With our rather large number of teeth and certain repeatedly appearing events in the background data, we were able to test three most commonly occurring life events in our material, vaccinations, ear infections and skin diseases, and offer new information on the effectiveness of these incidents in general to predict ALs.

Little is known about the causality of an AL and a certain life event based on previous studies. Vaccinations were unlikely to cause ALs according to our study. Birch and Dean (2014) reported that early childhood vaccinations and mother's cortisol inoculations during late gestation matched with a child's post- and prenatal ALs. However, this evidence was based on only two samples from twin babies born pre-term. Neither were ear infections strong enough stressors to engender ALs in our study. Teivens et al. (1996) studied otitis in their background data in a single individual and were not able to clearly associate it with ALs. Only few previous studies have conducted matching between life events and ALs. These are case reports of one (Skinner & Anderson, 1990; Schwartz et al., 2005), two (Birch & Dean, 2014) or several (n=19) (Teivens et al., 1996) individuals. Incidents in their background data vary, showing single events and diseases, such as transfer by ambulance between hospitals, gastro-intestinal problems, vomiting (twin babies, Birch & Dean, 2014), eye injury, hospital visits and transfer anaesthetized (female gorilla, Schwartz et al., 2005). By counting the exact enamel growth layers, these researchers were able to strongly prove the association between a single AL and a single event. However, our study with more material showed the poor likelihood of two common events, vaccinations, and ear infections, to result in AL formation.

AL identification can be somewhat problematic even when following the existing criteria by Goodman and Rose (1990). We found visible lines in enamel, but when magnified, the lines lacked the microstructural changes and sufficient length that are listed in the mentioned proposal. Previous researchers and groups have also expressed their concerns about the identification of ALs, such as “variation in distinctiveness on crown walls” (Birch and Dean, 2014) and “decision making in sufficient discernibility” (FitzGerald et al., 2006). Kierdorf et al. (2020) suggested minor changes, not filling the length criteria, to be recorded as 2nd order ALs when they investigated enamel with SEM, thus including all the enamel abnormalities in the group of ALs.

6.7 Strengths and limitations

The medical background data collected by professionals in firmly established Finnish child health care and health care systems, combined with a large deciduous tooth collection, are the strengths of this study. The FFC study offered a strong basis to analyse both enamel NNL and other ALs in relation to early childhood life. Limitations in this study were the uneven number of tooth types in the FDT collection in general. In addition, exact dates of life events would have given an opportunity to unite single events with exact ALs. The use of SEM could identify microstructural changes in the tooth enamel more accurately.

6.8 Future interests

A future interest is to investigate, how gestational and birth-related factors affect the NNL width in a collection of canines. The NNL width variation in bioarchaeological and forensic medicine studies is essential if it indeed indicates an individual’s pre-, peri- and postnatal circumstances. For forensic investigations, it is of interest to conduct a study to reveal the exact time when the NNL becomes visible. This would need a collection of samples from new-borns who passed away in differing hours to few days after birth. Additionally, a time window for prenatal enamel development and prenatal AL/ALs timing in relation to the NNL, also in relation to the medical context, could elucidate the open questions on NNL forming. There is a substantial need for a prospective population study in which the determined stress factors, preferably both physical and mental, clearly dated and defined, could be later linked to enamel microstructure.

Methodology-wise, the use of micro-CT could improve exploring the microstructural features in enamel. Scanning would not require laborious and skill-requiring laboratory work, especially considering the need for optimally planed ground sections.

7 Conclusions

The neonatal line exists in nearly all mature deciduous teeth and the line width is influenced by the duration of delivery. Ameloblasts are more responsive to stressors in the vertical third of the tooth crown height, both along the NNL in the form of engendering more microstructural changes, and in the occurrence of ALs. Ear infections or vaccinations do not as a rule engender disturbed enamel expressed as ALs. Up until now, and with future research, deciduous tooth enamel does indeed tell us of lived lives. The NNL, as an enamel birth mark, offers an opportunity for pre- and postnatal exploration.

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References

1. AlQahtani, S. J., Hector, M. P., and Liversidge, H. M. (2010). Brief communication: The London atlas of human tooth development and eruption. American Journal of Physical Anthropology, 142(3), 481–490. http://doi.org/10.1002/ajpa.21258
2. Antoine, D., Hillson, S. and Dean, C. (2009). The developmental clock of dental enamel: a test for the periodicity of prism cross-striations in modern humans and an evaluation of the most likely sources of error in histological studies of this kind. Journal of Anatomy, 214, 45-55. https://doi.org/10.1111%2Fj.1469-7580.2008.01010.x
3. Birch, W. and Dean, M. C. (2014). A method of calculating human deciduous crown formation times and of estimating the chronological ages of stressful events occurring during deciduous enamel formation. Journal of Forensic and Legal Medicine, 22, 127-144. https://doi.org/10.1016/j.jflm.2013.12.002
4. Boyde, A. (1989). Enamel. In A. Oksche & L. Vollrath (Eds.), Teeth, handbook of microscopic anatomy (Vol. V/6, pp. 309-473). Berlin, Germany: Springer.
5. Bromage, T. G. (1991). Enamel incremental periodicity in the pig-tailed macaque: A polychrome fluorescent labeling study of dental hard tissues. American Journal of Physical Anthropology, 86, 205-214.
6. Canturk, N., Atsu, S., Aka, P., and Dagalp, R. (2014). Neonatal line of fetus and infant teeth. An indicator of live birth and mode of delivery. Early Human Development, 90, 393–397. https://doi.org/10.1016/j.earlhumdev.2014.05.002
7. Central Statistical Office of Finland. Handbooks / Statistics Finland, 17 (in Finnish, English summary) Classification of Socio-economic Groups. Helsinki, Finland: Statistics Finland, 1989.
8. Dean, M. C. (1987a). The dental developmental status of six East African juvenile fossil hominids. Journal of Human Evolution, 16, 197-214.
9. Dean, M. C. (1987b). Growth layers and incremental markings in hard tissues; a review of the literature and some preliminary observations about enamel structure in Paranthropus boisei. Journal of Human Evolution, 16, 157-172.
10. Dean, M. (1989). The developing dentition and tooth structure in hominoids. Folia Primatol. 53, 160-176.
11. Dean, M. C., Beynon, A., Reid, D. and Whittaker, D. (1993a). A longitudinal study of tooth growth in a single individual based on long and short period incremental markings in dentine and enamel. International Journal of Osteoarchaeology, 3, 249-264.
12. Dean, M. C. (2006). Tooth microstructure tracks the pace of human life-history evolution. Proceedings of the Royal Society B, 273, 2799-2808. https://doi.org/10.1098/rspb.2006.3583
13. Demirjian, A., Goldstein H., and Tanner, J. (1973). A new system of dental age assessment. Human Biology Vol. 45, No. 2 (May 1973), pp. 211-227. https://www.jstor.org/stable/41459864
14. Eli, I., Sarnat, H., and Talmi, E. (1989). Effect of the birth process on the neonatal line in primary tooth enamel. Pediatric Dentistry, 11(3), 220–3.
15. FitzGerald, C. M. and Saunders, S. R. (2005). Test of histological methods of determining chronology of accentuated striae in deciduous teeth. American Journal of Physical Anthropology, 127, 277-290. https://doi.org/10.1002/ajpa.10442

16. FitzGerald, C., Saunders, S., Bondioli, L. and Macchiarelli, R. (2006). Health of infants in an Imperial Roman skeletal sample: Perspective from dental microstructure. American Journal of Physical Anthropology, 130, 179-189. https://doi.org/10.1002/ajpa.20275
17. Gamble, J. A., Boldsen, J. L. and Hoppa, R. D. (2017). Stressing out in medieval Denmark: An investigation of dental enamel defects and age at death in two medieval Danish cemeteries. International Journal of Paleopathology, 17, 52-66. https://doi.org/10.1016/j.ijpp.2017.01.001
18. Goodman, A. H. and Rose, J. C. (1990). Assessment of systemic physiological perturbations from dental enamel hypoplasias and associated histological structures. Yearbook of Physical Anthropology, 33, 59-110. https://doi.org/10.1002/AJPA.1330330506
19. Gustafson, G., and Gustafson, A. G. (1967). Microanatomy and histochemistry of enamel. In A. E. W. Miles (Ed.). Structural and chemical organization of teeth (Vol. II, pp. 75-134). New York, NY: Academic Press.
20. Gwinnett A. (1966). Histology of normal enamel. III. Phase contrast study. Journal of Dental Research, May-June, 865-869. https://doi.org/10.1177/00220345660450035901
21. Hasset, B., Humphrey, L. and Dean, C. (2017). Appearance of the neonatal line depends on gestational rather than delivery variables. Poster session presentation at the meeting of the 17th International Symposium on Dental Morphology & 2nd congress of International Association for Paleodontology, Book of Abstracts, 79, Bordeaux, France.
22. Hillson, S. (2002). Dental Anthropology. Cambridge, England: Cambridge University Press
23. Hillson, S. (2014). Teeth development in human evolution and bioarcheology. Cambridge, England: Cambridge University Press.
24. Jaakkola, S., Rautava, P., Saarinen, M., Lahti, S., Mattila, ML., Suominen, S., Räihä, H., Aromaa, M., Honkinen, PL., Sillanpää, M. (2013). Dental fear and sense of coherence among 18-yr-old adolescents in Finland. European Journal of Oral Sciences, 121, 247–251.
25. Kierdorf, H., Witzel, C., Bocaege, E., Richter, T. and Kierdorf, U. (2020). Assessment of physiological disturbances during pre- and early postnatal development based on microscopic analysis of human deciduous teeth from the Late Epipaleolithic site of Shubayqa 1 (Jordan). American Journal of Physical Anthropology, 1-15. https://doi.org/10.1002/ajpa.24156
26. Kodaka T., Sano T. and Higashi S. (1996). Structural and calcification patterns of the neonatal line in the enamel of human deciduous teeth. Scanning Microscopy, 10, 3: 737-744
27. Kronfeld, R. and Schour, I. (1939). Neonatal dental hypoplasia. Journal of American Dental Association 26 (January), 18-32.
28. Kurek, M., Borowska, B., Lubowiedzka-Gontarek, B., Rosset, I. and Żądzińska, E. (2020). Disturbances in primary dental enamel in Polish autistic children. Scientific Reports, 10, 12751. https://doi.org/10.1038/s41598-020-69642-3
29. Kurek, M., Żądzińska, E., Sitek, A., Borowska-Strugińska, B., Rosset, I. and Lorkiewicz, W. (2015). Prenatal factors associated with the neonatal line thickness in human deciduous incisors. HOMO - Journal of Comparative Human Biology, 66(3), 251–263. http://doi.org/10.1016/j.jchb.2014.11.001
30. Kurek, M., Żądzińska, E., Sitek, A., Borowska-Strugińska, B., Rosset, I. and Lorkiewicz, W. (2016). Neonatal line width in deciduous incisors from Neolithic, mediaeval and modern skeletal samples from north-central Poland. Annals of Anatomy, 203, 12-18. http://doi.org/10.1016/j.aanat.2015.02.006
31. Mahoney, P. (2011). Human deciduous mandibular molar incremental enamel development. American Journal of Physical Anthropology, 144, 204-214. https://doi.org/10.1002/ajpa.21386
32. Mahoney, P. (2012). Incremental enamel development in modern human deciduous anterior teeth. American Journal of Physical Anthropology, 147, 637-651. https://doi.org/10.1002/ajpa.22029
33. Mahoney, P. (2015). Dental fast track: Prenatal enamel growth, incisor eruption, and weaning in human infants. American Journal of Physical Anthropology, 156, 407-421. https://doi.org/10.1002/ajpa.22666

34. Mattila, ML., Rautava, P., Paunio, P., Ojanlatva, A., Hyssälä, L., Helenius, H., Sillanpää, M. (2001). Caries experience and caries increment in children at ten years of age. Caries Research, 35, 435–441.
35. Mattila, ML., Rautava, P., Paunio, P., Hyssälä, L., Helenius, H., Sillanpää, M. (2001). Impact of dental health care on dental health of children with long-term diseases: a nested case-control study. Journal of Clinical Dentistry, 12 (3), 77-82.
36. Mountain, R., Zhu, Y., Pickett, O., Lussier, A., Goldstein, J., Roffman, J., Bidlack, F. and Dunn, E. (2021). Association of maternal stress and social support during pregnancy with growth marks in children´s primary tooth enamel. Jama Network Open 4 (11). https://doi.org/10.1001/jamanetworkopen.2021.29129
37. Nava, A., Frayer, D. W. and Bondioli, L. (2019). Longitudinal analysis of the microscopic dental enamel defects of children in the Imperial Roman community of Portus Romae (necropolis of Isola Sacra, 2nd to 4th century CE, Italy). Journal of Archaeological Science: Reports, 23, 406-415. https://doi.org/10.1016/j.jasrep.2018.11.007
38. Norén, J. (1983). Enamel structure in deciduous teeth from low-birth-weight infants. Acta Odontologica Scandinavica, 41:6, 355-362. http://dx.doi.org/10.3109/00016358309162347
39. Paunio, P, Rautava, P., Helenius, H., Alanen, P., Sillanpää, M. (1993). The Finnish Family Competence Study: The relationship between caries, dental health habits and general health in 3-year-old Finnish children. Caries Research, 27, 154–160.
40. Rose, J. C. (1977). Defective enamel histology of prehistoric teeth from Illinois. American Journal of Physical Anthropology, 46, 439-46. https://doi.org/10.1002/ajpa.1330460309
41. Rose, J. C. (1979). Morphological variations of human enamel prisms within abnormal striae of Retzius. Human Biology, 51, 139-151.
42. Risnes, S. (1986). Enamel apposition rate and the prism periodicity in human teeth. Scandinavian Journal of Dental Research, 94, 394-404.
43. Risnes, S. (1990). Structural characteristics of staircase-type Retzius lines in human dental enamel analyzed by scanning electron microscopy. Anatomical Record, 226, 135–146.
44. Risnes, S. (1998). Growth tracks in dental enamel. Journal of Human Evolution, 35, 331-350. https://doi.org/10.1006/jhev.1998.0229
45. Robinson, C., Kirkham, J., Brookes, S. and Shore, R. (1995). Chemistry of mature enamel. Dental enamel, formation to destruction (pp. 167-191). Boca Raton, USA: CRC Press.
46. Rushton, M. (1933). On the fine contour lines of the enamel of milk teeth. Dental Record, 53, 170–1.
47. Rythén, M., Norén, J., Sabel, N., Steiniger, F., Niklasson, A., Hellström, A. and Robertson, A. (2008). Morphological aspects of dental hard tissues in primary teeth from preterm infants. International Journal of Paediatric Dentistry, 18: 397–406. https://doi.org/10.1111/j.1365-263X.2008.00928.x
48. Sabel, N., Johansson, C., Kühnisch, J., Robertson, A., Steiniger, F., Norén, J. G. and Nietzsche, S. (2008). Neonatal lines in the enamel of primary teeth—A morphological and scanning electron microscopic investigation. Archives of Oral Biology, 53(10), 954–963. https://doi.org/10.1016/j.archoralbio.2008.05.003
49. Schour, I. (1936). The neonatal line in the enamel and dentin of the human deciduous teeth and first permanent molar. The Journal of the American Dental Association, 23(10):1946– 1955. https://doi.org/10.14219/jada.archive.1936.0277
50. Schwartz, G. T., Reid, D. J., Dean, M. C. and Zihlman, A. L. (2006). A faithful record of stressful life events recorded in the dental development record of a juvenile gorilla. International Journal of Primatology, 27, 1201-19. https://doi.org/10.1007/s10764-006- 9051-2
51. Shellis, R. P. (1984). Variations in growth of the enamel crown in human teeth and a possible relationship between growth and enamel structure. Archives of Oral Biology, 29, 697-705. https://doi.org/10.1016/0003-9969(84)90175-4

52. Sidaly, R., Risnes, R., Khan, Q., Stiris, T. and Sehic, A. (2015). The effect of hypoxia on the formation of mouse incisor enamel. Archives of Oral Biology, 60, 1601-1612.
53. Sillanpää, M. and Rautava, P. (1989). The Finnish Family Competence Study: Knowledge of childbirth of nulliparous women seen at maternity health care clinics. Journal of Epidemiology and Community Health, 43, 253-260. https://doi.org/10.1136%2Fjech.43.3.253
54. Sillanpää, M., Camfield, P., Camfield, C., Aromaa, M., Helenius, H., Rautava, P., Hauser, A. (2008). Inconsistency between prospectively and retrospectively reported febrile seizures. Developmental Medicine & Child Neurology, 50, 25–28.
55. Skinner, M. and Anderson, G. (1991). Individualization and enamel histology: A case report in forensic anthropology. Journal of Forensic Sciences 36: 3, 939–948.
56. Skinner, M. and Dupras, T. (1993). Variation in birth timing and location of the neonatal line in human enamel. Journal of Forensic Science, 38 (6), 1383–1390.
57. Sourander, A., Aromaa, M., Pihlakoski, L., Haavisto, A., Rautava, P., Helenius, H., Sillanpää, M. (2006). Early predictors of deliberate self-harm among adolescents. A prospective follow-up study from age 3 to age 15. Journal of Affective Disorders; 93, 87–96.
58. Teivens, A., Mörnstad, H., Norén, J. G. and Gidlund, E. (1996). Enamel incremental lines as recorders for disease in infancy and their relation to the diagnosis of SIDS. Forensic Science International, 81, 175-183. https://doi.org/10.1016/S0379-0738(96)01982-2
59. Weber, D.F. and Eisenmann, D.R. (1971). Microscopy of the neonatal line in developing human enamel. American Journal of Anatomy, 132, 375-391.
60. Weber, D.F., Eisenmann, D.R. and Glick, P.L. (1974). Light and electron microscopic studies of Retzius lines in human cervical enamel. American Journal of Anatomy, 141, 91– 104.
61. Whittaker, D. K. and Richards, D. (1978). Scanning electron microscopy of the neonatal line in human enamel. Archives of Oral Biology, 23(1), 45–50. https://doi.org/10.1016/0003-9969(78)90052-3
62. Willems, G., Van Olmen, A., Spiessens, B. and Carels, C. (2001). Dental age estimation in Belgian children: Demirjian's technique revisited. Journal of Forensic Sciences, 46(4):893- 5.
63. Wilson D.F. and Shroff F.R. (1970). The nature of the striae of Retzius as seen with the optical microscope. Australian Dental Journal, 15, 162-171.
64. Witzel, C., Kierdorf, U., Schultz, M. and Kierdorf, H. (2008). Insights from the inside: Histological analysis of abnormal enamel microstructure associated with hypoplastic enamel defects in human teeth. American Journal of Physical Anthropology, 136, 400-414. https://doi.org/10.1002/ajpa.20822.
65. Zanolli, C., Bondioli, L., Manni, F., Rossi, P. and Macchiarelli, R. (2011). Gestation length, mode of delivery, and neonatal line-thickness variation. Human Biology, 3 (6), 695-713. http://dx.doi.org/10.3378/027.083.0603
66. Żądzińska, E., Kurek M., Borowska-Strugińska, B., Lorkiewicz, W., Rosset, I. and Sitek, A. (2013). The effect of the season of birth and of selected maternal factors on linear enamel thickness in modern human deciduous incisors. Archives of Oral Biology, 58, 951-963
67. Żądzińska, E., Lorkiewicz, W., Kurek, M. and Borowaka-Struginska, B. (2015). Accentuated lines in the enamel of primary incisors from skeletal remains: a contribution to the explanation of early childhood mortality in a medieval population from Poland. American Journal of Physical Anthropology, 157, 402-410.

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