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ULTRASTRUCTURE OF DENTIN MATRIX IN HERITABLE DENTIN DEFECTS

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Abstract

Heritable dentin defects form a group of diseases which exclusively affect dentin among the various dental tissues. While one type is associated with the generalized connective tissue disorder, osteogenesis imperfecta, other types occur as single traits. The clinical manifestations of the dentin defects vary from insignificant to severe enough to cause aesthetical and functional failure of the teeth. Scanning and transmission electron microscopic studies, reviewed in this paper, have markedly clarified the ultrastructure of the aberrant dentin matrix. Both similar and different changes seem to occur in the various forms of heritable dentin defects. Abnormalities in the appearance and organization pattern of collagen fibers in the defective dentin partly resemble those observed in skin in generalized connective tissue diseases. The similarity of ultrastructural findings in dentin defects, which are currently classified as distinct entities, and even in diseases affecting other tissues, could be related to the complicated interactions between the extracellular matrix macromolecules. Thus, many of the changes observed may be secondary in nature. Ultrastructural studies can help us to understand the pathogenesis of the different types of heritable dentin defects as well as aid in diagnostics and classification of these diseases.

Key Words: Human teeth, heritable dentin defects, dentin dysplasia, dentinogenesis imperfecta, osteogenesis imperfecta, extracellular matrix, collagen, histology, scanning electron microscopy, transmission electron microscopy.

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Introduction

Heritable human dentin defects are diseases which affect the (ecto)mesenchyme-derived dentin among the various dental tissues. Notably, the enamel, which is ectodermal in origin, appears normal. These disorders have been divided into two major categories: dentin dysplasia (DD) and dentinogenesis imperfecta (DI). Type I DI is the dental manifestation of the generalized connective tissue disorder osteogenesis imperfecta (OI), whereas the two other types of DI, as well as both types of DD, appear to affect dentin solely (Shields et al., 1973). Table 1 presents the characteristic features of different types of DD and DI. As these diseases share many clinical and radiographic features, and the expression, especially in the permanent teeth, may be mild, it is often difficult to make the correct diagnosis.

Analyses with scanning electron microscopy (SEM) and transmission electron microscopy (TEM) have confirmed the histological findings that the dentinal tubules are irregular and sparse in the different types of DI (Levin et al., 1980, 1983; Waltimo et al., 1994) as well as in the abnormal parts of dentin in type II DD (Melnick et al., 1977; Waltimo et al., 1991). Type I DD, on the other hand, is characterized by the aberrant orientation of the tubules (Sauk et al., 1972; Wesley et al., 1976). Moreover, electron microscopic studies have revealed structural abnormalities beyond the level of detection of other methods. As indicated by TEM, a haphazard organization pattern and varied size of collagen fibers are also common findings (Herold, 1972; Waltimo et al., 1991, 1994), whereas vesicular structures, unravelled collagen fibers and hyperfibers have been observed in type I DI only (Waltimo, 1994; Waltimo and Lukinmaa, unpublished results).

In some patients with OI, a variety of ultrastructural changes are seen in dentin, whereas other patients appear to have normal teeth, independently of the general severity of the disease. Thus, in both DI associated with OI, in which type I collagen is genetically defective, and in dentin defects inherited as single traits, where the gene defect has not been specified, ultrastructural studies can be expected to clarify the so far poorly understood

Table 1. Characteristic features of the different types of dentin dysplasia (DD) and dentinogenesis imperfecta (DI)[*].

Feature	Type of defect			
	DD-I	DD-II	DI-I	DI-II
Associated with osteogenesis imperfecta	-	-	+ +	-
Inherited as a single trait	+ +	+ +	-	+ +
Clinical findings				
Primary teeth discolored	-	+ +	+ +	+ +
Permanent teeth discolored	-	-	+	+ +
Loose teeth	+ +	-	-	-
Rapid attrition in permanent teeth	-	-	+	+ +
Radiographic findings				
Bulbous crowns	-	-	+ +	+ +
Short roots	+ +	-	+	+
Obliteration of pulp chambers in primary teeth	+ +	+ +	+ +	+ +
Obliteration of pulp chambers in permanent teeth	-	-	+	+ +
Crescent-shaped pulp chambers in permanent teeth	+ +	-	-	-
Thistle-tube shaped pulp chambers in permanent teeth	-	+ +	+	-
Pulp stones in permanent teeth	+	+ +	+	-
Periapical radiolucencies	+ +	-	+	+
Histological findings				
Abnormal dentin in primary teeth	+ +	+ +	+ +	+ +
Abnormal coronal dentin in permanent teeth	-	-	+	+ +
Abnormal radicular dentin in permanent teeth	+ +	+ +	+	+ +

- = usually not present;   + = sometimes present;   + + = usually present

[*] Freely modified after Shields *et al.* (1973).

pathogenesis of the disorders. As dentin, unlike bone, is not remodeled, any major disturbance in odontoblast function has definite morphological consequences, by which the time, and sometimes even the nature, of the damage can be determined. In this paper, we review the ultrastructural findings in dentin affected by heritable dentin defects, and discuss their pathogenetic implications.

Formation and Structure of Normal Dentin Matrix

Dentin, like bone, is a mineralized connective tissue. Odontoblasts deposit the organic matrix of dentin, which subsequently becomes mineralized (Fig. 1). These cells differentiate from the neural crest-derived cells of the dental papilla as a result of inductive epithelial-mesenchymal interactions involving a variety of transcription factors, growth factors as well as structural molecules (Lesot *et al.*, 1981; Ruch, 1987; Thesleff *et al.*, 1991; Bègue-Kirn *et al.*, 1992; Jowett *et al.*, 1993; Vainio *et al.*, 1993; Heikinheimo, 1994). Dentinogenesis starts at the cuspal/incisal region and proceeds in an apical direction; at the same time, new odontoblasts
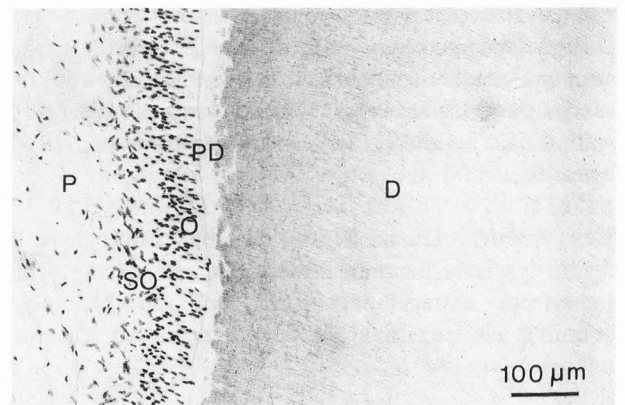

Figure 1. Histological appearance of the pulp-dentin complex of a (demineralized) normal permanent tooth. A confluent layer of odontoblasts (O) encompasses the pulp (P) composed of loose-textured connective tissue. Also, the subodontoblastic cell layer (SO) is clearly outlined. A faint, regular tubular pattern is discernable in predentin (PD) and dentin (D), which are demarcated by a globular mineralization front. Hematoxylin and eosin stain. Bar = 100 µm.
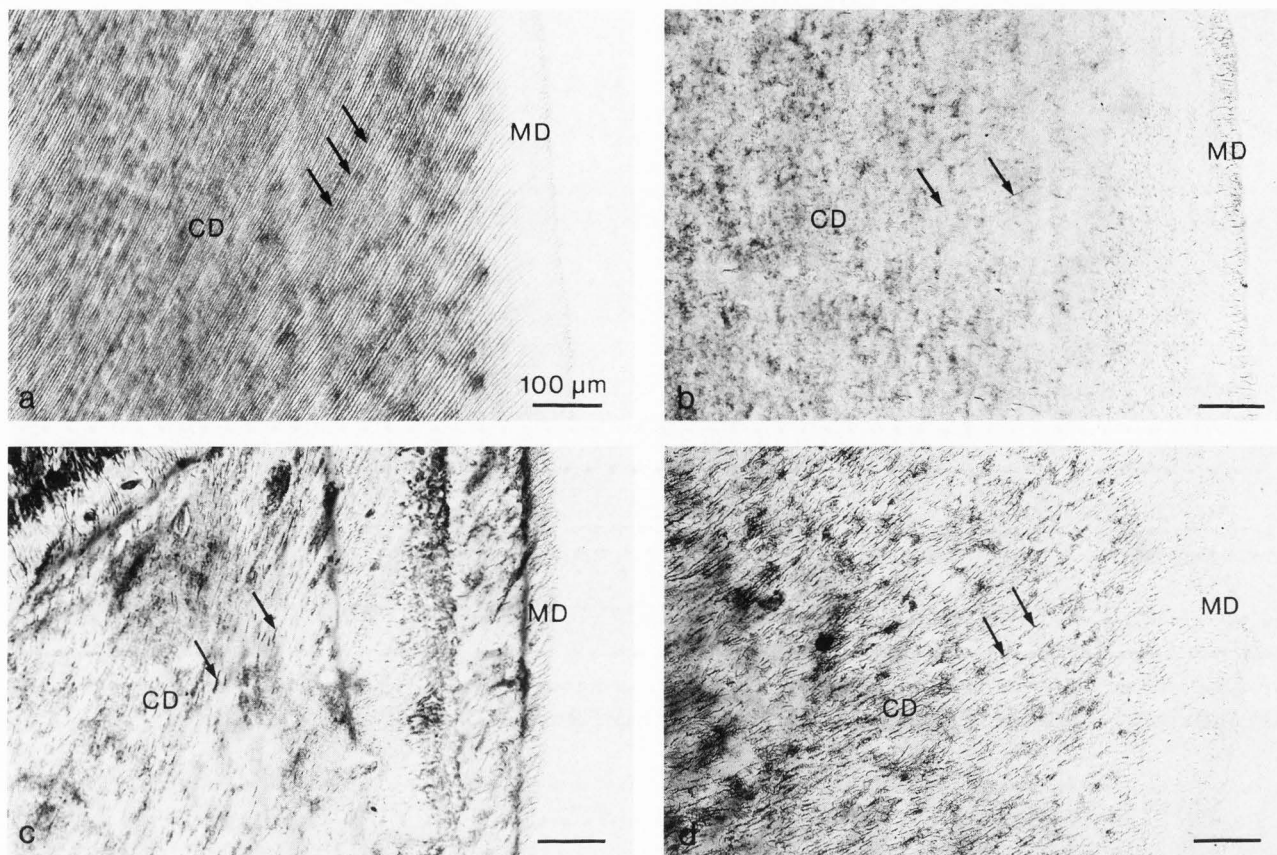
Figure 2. Histological appearance of normal (**a**) and diseased (**b-d**) dentin of deciduous (**a-c**) and permanent (**d**) teeth (CD: circumpulpal dentin; MD: mantle dentin). In contrast to normal dentin (**a**), where the regular dentinal tubules (arrows) extend to the dentin enamel junction, the tubular pattern is aberrant in dentin affected with dentin dysplasia type II (**b**), and dentinogenesis imperfecta associated with osteogenesis imperfecta (**c**) and occurring as a single trait (**d**). Note the lamellar structure of dentin in (**c**) and the fairly regular tubular pattern in mantle dentin in (**b**) and (**c**). Enamel (matrix) has been lost during demineralization. Schmorl's picric acid and thionin stain. Bars = 100 µm.

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differentiate further apically.

The outermost layer, or mantle dentin, covers the bulk of circumpulpal dentin (Fig. 2a). These are both mineralized, in contrast to the thin layer of predentin, which lines the pulpal cavity even after the completion of tooth development (Fig. 1). The odontoblast processes are situated in dentinal tubules (Fig. 3a), which make up the most striking histological and ultrastructural feature of dentin (Figs. 2a and 3a-3c). The width of the tubules decreases towards the dentin-enamel junction (Maniatopoulos and Smith, 1983; Sögaard-Pedersen et al., 1990) due to gradual thickening of the peritubular dentin (Fig. 3). Because of technical problems associated with fixation and demineralization of dentin, it has been difficult to determine how far the odontoblast processes extend (for review, see Holland, 1985; Frank and Steuer, 1988). The cellular processes send lateral branches through which the neighbouring odontoblasts may make contact (Maniatopoulos and Smith, 1983;

White et al., 1986).

The mineralized intertubular dentin constitutes the main part of dentin, the organic matrix of which is composed of a compact web of collagen fibers. Most fibers are oriented parallel or at an acute angle to the incremental pattern (Sögaard-Pedersen et al., 1990) (Fig. 3). The dentinal tubules are lined by highly mineralized peritubular dentin which has a sparse organic matrix (White et al., 1986; Yoshiyama et al., 1990). Odontoblast processes are occasionally seen intratubularly (Fig. 3a), and nerves are observed rarely. The dentinal tubules often appear empty (Fig. 3b), but they can also contain collagen fibers (Thomas and Carella, 1983, 1984), amorphous or granular material (White et al., 1986), or an electron-dense sheath, referred to as the lamina limitans (Thomas and Carella, 1983, 1984) (Fig. 3c). In undemineralized sections, the tubules may be occluded by mineral crystals (Yoshiyama et al., 1990).
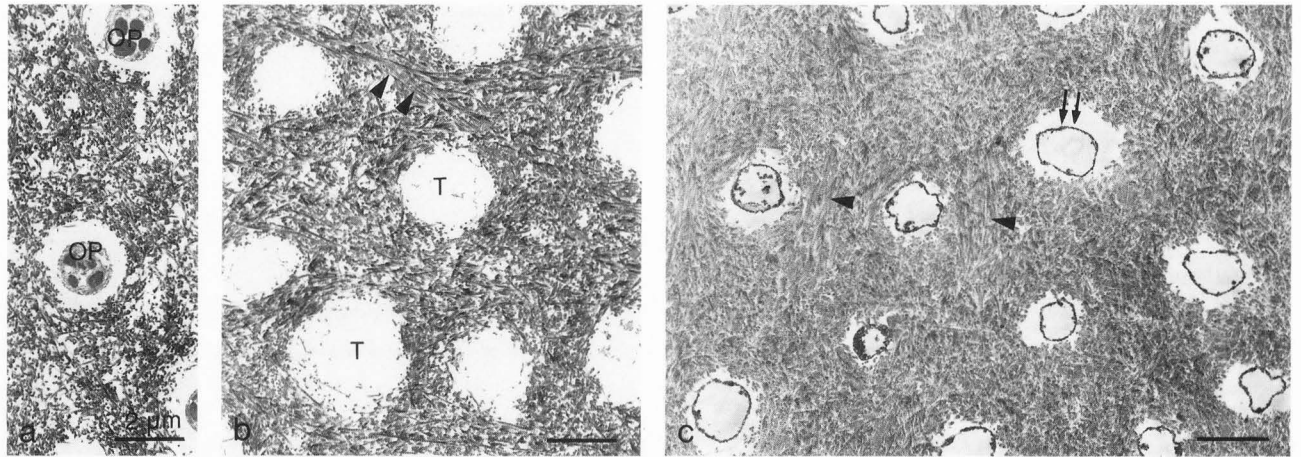
Odontoblasts not only produce the dentin matrix,

Figure 3. Transmission electron micrographs of dentin of normal primary teeth. (a) Sections of dentin close to the pulp contain occasional odontoblast processes (OP) within the cross-sectioned dentinal tubules. This tooth was extracted for orthodontic reasons and still contained pulp tissue. In the middle third of dentin, the tubules (T) appear empty (b); whereas, more externally, sheath-like structures (arrows) line the tubules (c). Many of the intertubular collagen fibers are arranged parallel to the incremental pattern (arrowheads in b, c). With increasing distance from the pulp, the diameter of the tubules and their number per unit area decline. At the same time, the intertubular dentin matrix becomes more tightly packed with collagen fibers which, in turn, become thicker. Bars = 2 μm.

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which is conducive of mineralization, but they also act as a route of transport and site of accumulation of the calcium ions, and produce matrix vesicles. The matrix vesicles, rich in alkaline phosphatase (Orams and Snibson, 1982), may be budded off the cells, and they probably serve as crystallization centers during the early stages of dentinogenesis or mantle dentin formation (for review, see Holland, 1985). Matrix vesicles are present in mantle dentin, but they have not been seen in the circumpulpal dentin (for review, see Bonucci, 1984).

Compared to the non-mineralized predentin, the organic matrix of the mineralized circumpulpal dentin contains collagen fibers that have a larger diameter (about 100 nm) and are more tightly packed (Sögaard-Pedersen *et al.*, 1990). They show the typical cross-striation pattern and round cross-section. It is atypical for collagen fibers to form bundles in normal dentin (Sögaard-Pedersen *et al.*, 1990) (Fig. 3). The fibers are mainly composed of type I collagen (for reviews, see Butler, 1984; Linde and Goldberg, 1993). Immunoreactivities of collagen types III (Nagata *et al.*, 1992; Lukinmaa *et al.*, 1993; Waltimo *et al.*, 1994), V (Bronckers *et al.*, 1986) and VI (Becker *et al.*, 1986) have also been observed in rodent and human dentin. Dentin also contains non-collagenous proteins, such as: phosphoproteins, γ-carboxyglutamate-containing proteins, glycoproteins, proteoglycans and serum proteins (for review, see Linde and Goldberg, 1993).

Heritable Dentin Defects

Classification

Abnormalities of dentin can be divided into those which primarily affect the organic matrix, including the disorders known as heritable dentin defects, and into those which lead to defective mineralization. These categories partially overlap, since normal structure of the matrix is a prerequisite for proper mineralization. The heritable dentin defects have been classified into two types of dentin dysplasia (DD) and three types of dentinogenesis imperfecta (DI) on the basis of clinical, radiographic, and histological criteria. Types I and II DD as well as types II and III DI affect dentin exclusively, whereas type I DI is the dental defect of osteogenesis imperfecta (OI) (Shields *et al.*, 1973). Yet, there are defects which do not fit this classification (for review, see Witkop, 1975). The defects affecting dentin solely, as well as OI in most families, are inherited as autosomal dominant traits. Type II DI, which affects about 1:8000 subjects, is among the most common dominantly inherited aberrations in man (Witkop, 1975). Of the patients with OI, 10-50% have been reported to have the dentin defect (Smith *et al.*, 1983; Lukinmaa *et al.*, 1987).

Technical aspects of ultrastructural studies

Naturally shed primary teeth are readily available,

whereas permanent teeth can be studied only when extracted for therapeutic reasons. However, the manifestations of the diseases in primary and permanent teeth may differ. The organic dentin matrix can either be studied in the narrow predentin zone or by examining the dentin after demineralization. Because of physiological resorption, even normal, naturally exfoliated primary teeth are usually lacking predentin. Furthermore, pulp chambers of the affected primary and permanent teeth are usually more or less obliterated, and predentin is thus not available for examination. Pulpal obliteration and the sparsity of dentinal tubules also cause difficulties in fixation. To overcome the poor penetration of the fixative, high concentrations of glutaraldehyde (2-5%) and prolonged fixation times (overnight to 24 hours) have been used. Yet, even in these conditions, the ultrastructure as well as antigenicities of at least various collagen types are preserved as indicated by immuno-TEM (Waltimo et al., 1994).

It is evident that odontoblast processes of normal dentin can be studied in greatest detail when the teeth are non-demineralized (Frank and Steuer, 1988). Teeth affected with heritable dentin defects have also been studied by SEM and TEM without demineralization (Kerebel, 1975; Skinner et al., 1978; Levin et al., 1980, 1982, 1983; Melnick et al., 1980; Kerebel et al., 1981; Jasmin and Clergeau-Guerithault, 1984), and by SEM after a short treatment of the specimen surface with ethylenediaminetetra-acetic acid (EDTA) (Sauk et al., 1972; Wesley et al., 1976). In non-demineralized samples, the tubular pattern and, notably, disturbances in mineralization, can be analyzed, but little information is obtained on the ultrastructure of the dentin matrix. Thorough demineralization, on the other hand, leads to extraction of water-soluble macromolecules, for example, proteoglycans, from the dentin matrix, which thereafter consists of a more or less stripped network of cross-linked collagen. Partially, this can be avoided by using EDTA in an organic instead of an aqueous solvent (Scott and Kyffin, 1978)

For our own studies on teeth affected by heritable dentin defects, described in this paper, primary and/or permanent teeth were obtained from patients with type II DD, type II DI (inherited as a single trait) and from patients classified as having types IB and IVB OI (Sillence, 1988). Control teeth were from subjects without any known developmental defects of teeth. The primary teeth were naturally shed, and the permanent teeth were extracted for valid therapeutical reasons. The teeth were fixed with 10% neutral buffered formalin or with ethanol, and demineralized with aqueous EDTA at 4°C for about two months. For light microscopy, the teeth were conventionally embedded in paraffin and cut into 5 μm-sections, which were either stained with hematoxy-

lin and eosin or by Schmorl's picric acid and thionin method. For TEM, small blocks were cut from the demineralized dentin. The blocks were fixed with glutaraldehyde (4%; overnight) and tannic acid (4%; 2 hours), and routinely prepared into ultrathin sections which were treated with uranyl acetate and lead citrate. The sections were examined in a JEOL JEM-1200 EX TEM at the Department of Electron Microscopy, University of Helsinki.

Structure of the Abnormal Dentin Matrix

Type I dentin dysplasia (DD)

The affected teeth are clinically normal or slightly discolored, but have short roots. The pulp chambers of the primary teeth are completely obliterated, but in the permanent teeth, a crescent-shaped remnant of the pulp chamber persists. Periapical radiolucencies and pulp stones are seen in radiographs (Sauk et al., 1972; Shields et al., 1973; Steidler et al., 1984). Histologically, the outermost layer of dentin is normal in both dentitions. More apically, the dentin shows atubular areas in primary teeth, and a cascade-like pattern of organization in the permanent teeth (Shields et al., 1973; Wesley et al., 1976).

SEM studies have shown that the cascade-like formations in dentin of permanent teeth contain tubules which appear normal, but are aberrantly oriented (Sauk et al., 1972; Wesley et al., 1976). The dentinal tubules of affected primary teeth are thinner and fewer in number than those of normal deciduous teeth (Melnick et al., 1980). It has been hypothesized that cells, prematurely disintegrating from the abnormal epithelial root sheath of Hertwig, could repeatedly induce differentiation of the mesenchymal cells of the developing root into dentin-forming cells. Thus, according to this theory, the primary defect would reside in the epithelial cells (Sauk et al., 1972; Witkop, 1989).

Type II DD

The primary teeth are often discolored whereas the permanent teeth usually appear normal. The pulp chambers of the primary teeth are completely obliterated, but those of the permanent teeth exhibit a characteristic, thistle-tube form, and may be filled with denticles (Shields et al., 1973; Steidler et al., 1984; Ranta et al., 1990, 1993). Histologically, the dentinal tubules of primary teeth are sparse and irregular (Ranta et al., 1990) (Fig. 2b). In the permanent teeth, there is an abrupt change from normal coronal dentin to abnormal radicular dentin, where an irregular tubular pattern and pathological, canal-like structures are observed (Steidler et al., 1984; Ranta et al., 1990, 1993). SEM and TEM studies on primary teeth show that the dentin is structurally highly disorganized, and it contains only few, thin
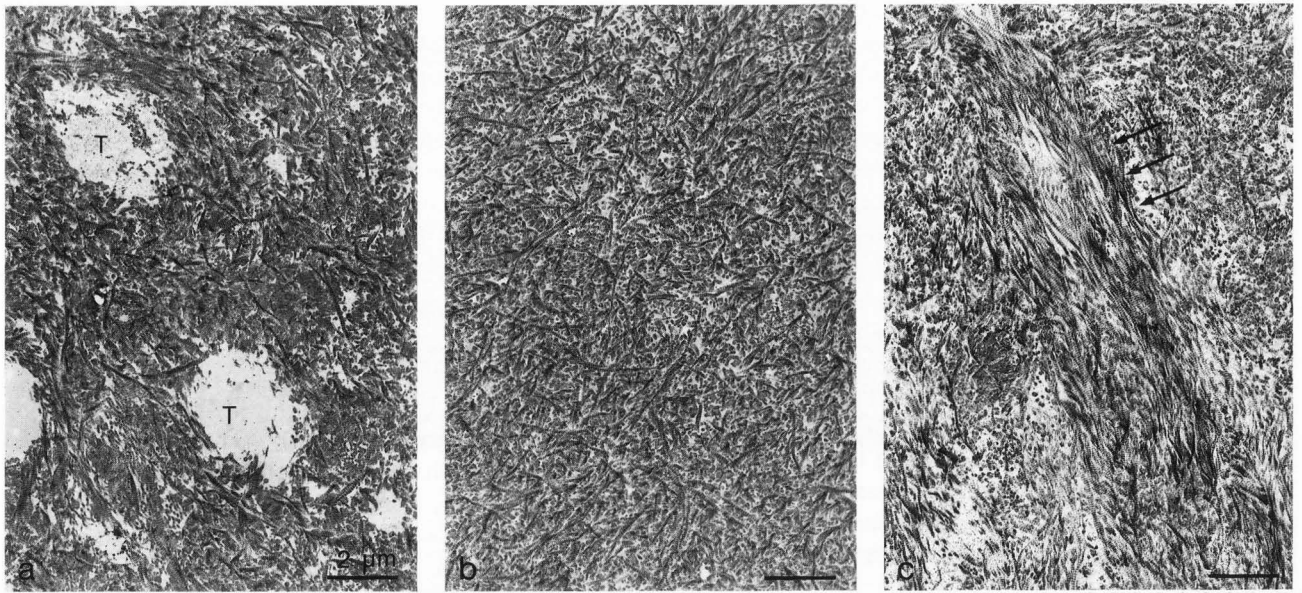
Figure 4. Transmission electron microscopic appearance of dentin of primary teeth from two patients representing different families with type II dentin dysplasia. (**a**) The tubules (T) in the affected dentin are irregular in size and shape. In the atubular areas, predominating in dentin matrix, the collagen fibers either form a haphazard meshwork (**b**) or large bundles (arrows) (**c**). Bars = 2 μm.

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tubules (Melnick *et al.*, 1977; Jasmin and Clergeau-Guerithault, 1984; Waltimo *et al.*, 1991) (Fig. 4). The coronal dentin of permanent teeth was normal in TEM as well, whereas the nearly atubular radicular dentin contains both haphazardly oriented collagen fibers and also thick, sometimes curvy, fiber bundles. The diameter of the fibers varied from abnormally thin to thicker than normal (Waltimo *et al.*, 1991).

Dentinogenesis imperfecta (DI) inherited as a single trait (types II and III)

Whereas type I DI is the dental manifestation of OI, types II and III appear to affect dentin solely (Shields *et al.*, 1973). Whether type III DI, described in a triracial isolate in Brandywine, Maryland (Hursey *et al.*, 1956; Witkop *et al.*, 1966), is a genetically distinct entity (Shields *et al.*, 1973; Levin *et al.*, 1983) or a variant of type II DI (Heimler *et al.*, 1985; Witkop, 1989) is not quite clear.

Both primary and permanent teeth affected by type II DI appear discolored and translucent. Although normal in structure, the enamel tends to crack off, which leads to rapid attrition of the underlying, abnormally soft dentin. Radiographic findings include bulbous crowns, cervical constrictions and short roots. While the pulp chambers gradually obliterate in type II DI, they may remain abnormally large in type III (Witkop and Rao, 1971; Shields *et al.*, 1973). Histologically, the first-formed layer of dentin is often normal, but the bulk of

(inner) dentin contains coarse and extensively branched tubules, which are few in number (Roberts and Schour, 1939; Waltimo *et al.*, 1994) (Fig. 2d), as well as abnormal, canal-like structures (Wright and Gantt, 1985).

Studies with SEM show that the dentinal tubules are scarce and of varied size in type II DI (Kerebel, 1975; Levin *et al.*, 1983; Wright and Gantt, 1985), and even more sparse in type III DI (Levin *et al.*, 1983). TEM studies, in addition, show that the orientation of the collagen fibers is aberrant (Kerebel, 1975; Kerebel *et al.*, 1981), and their diameter may either be increased (Herold, 1972) or decreased (Waltimo *et al.*, 1994). Atypical fibrillar structures in dentin matrix have also been documented (Waltimo *et al.*, 1994). In general, the matrix consists of a monotonic meshwork of collagen fibers of normal or slightly reduced diameter. Dentinal tubules are uniformly present in occasional areas only and are seldom seen in sections from the inner dentin (Fig. 5). Increased immunoreactivity of type III collagen has been observed in the dentin matrix in type II DI (Sauk *et al.*, 1980; Waltimo *et al.*, 1994). As opposed to normal dentin (Lukinmaa and Waltimo, 1992), type VI collagen was found in finely fibrillar material in immuno-TEM (Waltimo *et al.*, 1994).

The gene defect of DI occurring as a single trait has been mapped to the long arm of human chromosome 4 (Ball *et al.*, 1982; Boughman *et al.*, 1986; Gusella *et al.*, 1986; Crall *et al.*, 1988), but it has not been specified so far. At least one type of phosphophoryn,
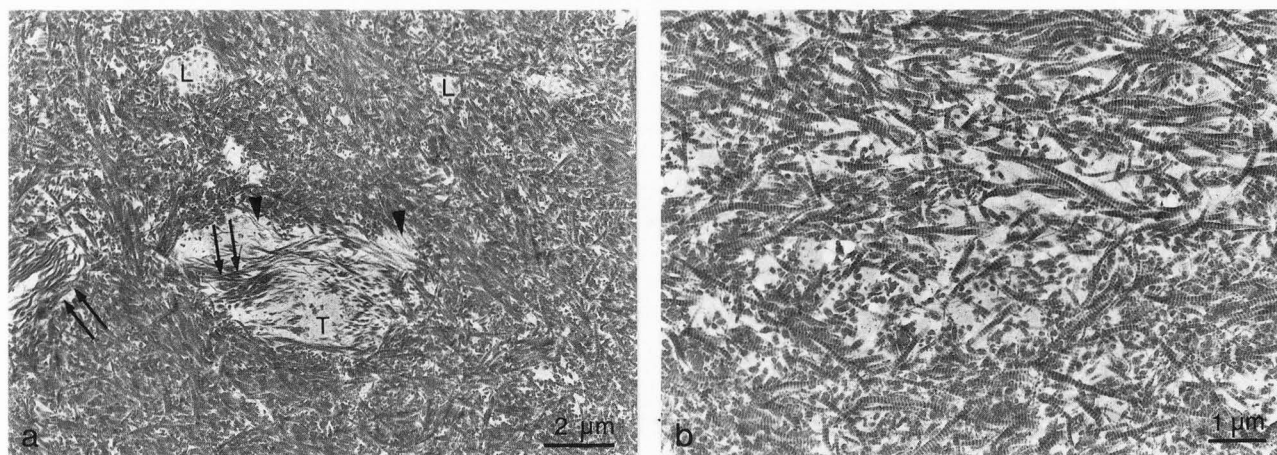
Figure 5. Transmission electron microscopic appearance of dentin of primary teeth from a patient with type II dentinogenesis imperfecta (inherited as a single trait). **(a)** The dentinal tubules (T) are poorly contoured and of varied size and shape. The abnormal, intratubular collagen fiber bundles (arrows) also display thin fibrillar structures (arrowheads). The abundant small openings (L) in the tubular areas probably correspond to the numerous lateral branches observed by light microscopy. **(b)** In the predominating atubular areas, the collagen fibers are mostly arranged haphazardly. Bars = 2 μm (a); 1 μm (b).

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belonging to the dentin-specific phosphoproteins, and reported to be lacking from the affected dentin (Takagi *et al.*, 1983; Takagi and Sasaki, 1986, 1988), has been excluded as a candidate gene (MacDougall *et al.*, 1992, 1994).

Dentinogenesis imperfecta associated with osteogenesis imperfecta (type I DI)

Osteogenesis imperfecta (OI) results from family-specific mutations in both genes coding for the pro-α chains of type I collagen (Sykes *et al.*, 1986; Byers *et al.*, 1992). The patients suffer from fragile and deformed bones, and they may also have hearing impairment, cardiovascular abnormalities, blue sclerae, and abnormal dentin. Depending on the site and type of the gene mutation, the severity of the disease varies from mild to lethal. The dentin defect (type I DI) seems, however, to occur independently of the severity of the general manifestations of the disease, except that the patients with the heterogenous type I OI (Levin *et al.*, 1980; Sillence, 1988), who also have DI, are usually more severely affected (Paterson *et al.*, 1983). On the other hand, children who died shortly after birth because of OI have had ultrastructurally either abnormal (Godfrey, 1973) or normal teeth (Levin *et al.*, 1982).

Types I and II DI appear to share many features. The primary teeth of patients with DI associated with OI are commonly more severely affected than are the permanent teeth in which the signs of DI may be virtually lacking (Sillence *et al.*, 1979a, b; Lukinmaa *et al.*, 1987). Also in primary teeth, the abnormality can be so

mild as to be disclosed only by histological and ultrastructural studies (Waltimo and Lukinmaa, unpublished observations). The histological appearance of teeth affected by OI markedly varies, but irregularly scattered dentinal tubules and lamellar dentin matrix are characteristic features (Fig. 2c).

Sparse, thin, and irregular dentinal tubules are consistent findings in SEM (Skinner *et al.*, 1978; Levin *et al.*, 1980). The most versatile features revealed by TEM are those found in type I DI associated with OI. These include vesicular structures, remarkably thick hyperfibers (thus far described in one patient only), and uncoiled collagen fibers (Waltimo, 1994; Waltimo *et al.*, 1994; Waltimo and Lukinmaa, unpublished observations) (Figs. 6b-6d). In general, the dentin matrix contains both normal-appearing tubular areas and abnormal areas where the ultrastructure markedly varies. The tubules may be irregular in shape and diameter, sparse or even absent. The collagen fibers may be tightly packed or scarce (Fig. 6a), and their organization pattern varies from a haphazard meshwork to abnormal, parallel alignment, yet distinct from bundle formation (Takagi *et al.*, 1980; Ranta *et al.*, 1993; Waltimo, 1994) (Fig. 6d). The size of the collagen fibers may be normal (80-100 nm), occasionally increased (Ranta *et al.*, 1993) or often reduced to 40-60 nm (Waltimo *et al.*, 1994).

The abundant thin fibers, seen in dentin matrix (Fig. 6d), are suggestive of the presence of type III collagen. Reactivity of type III collagen, which was previously observed at the light microscopic level in dentin in DI (Sauk *et al.*, 1980; Lukinmaa, 1988), was shown to be
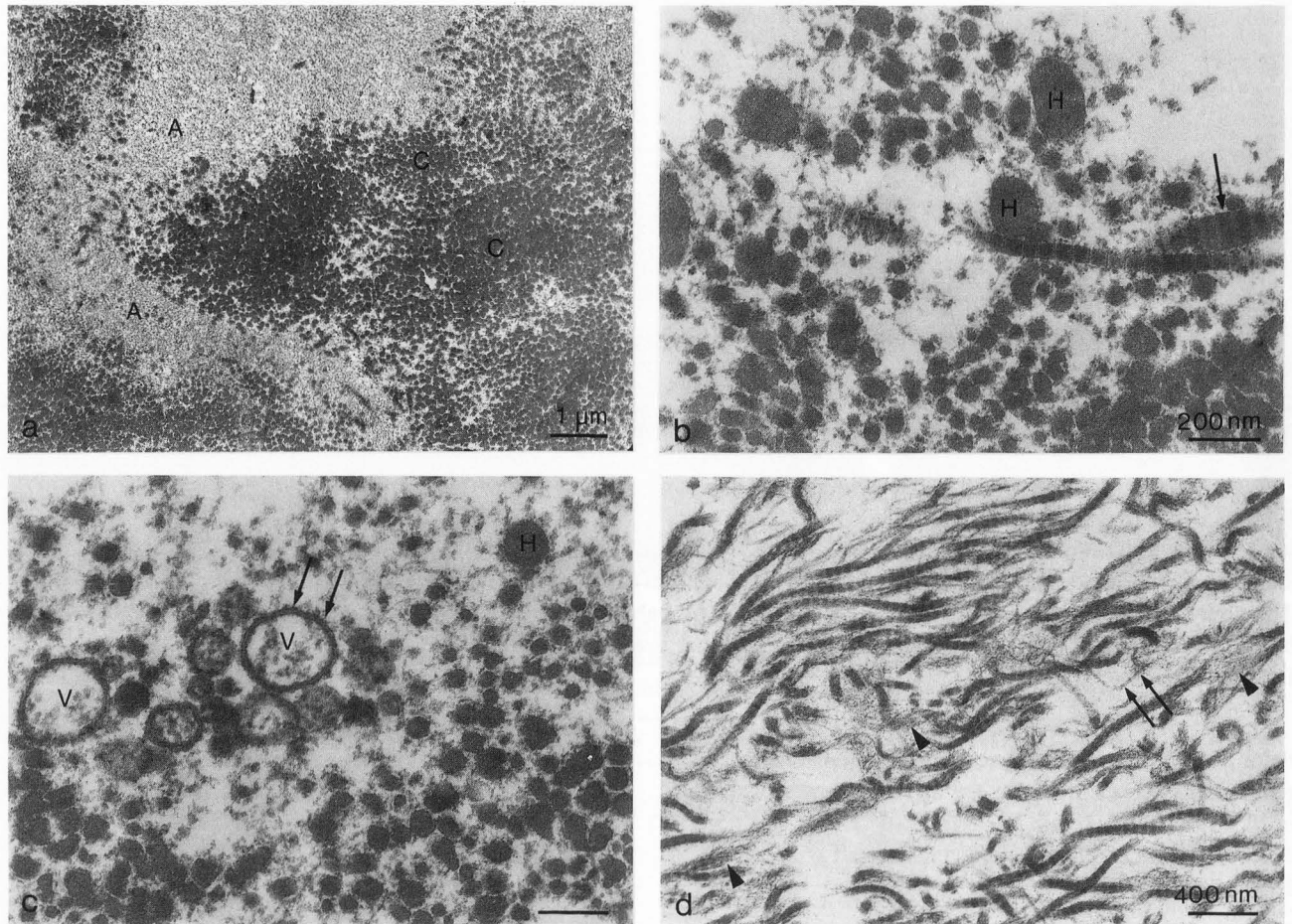
Figure 6. Transmission electron microscopic appearance of dentin of primary teeth from two patients representing type I dentinogenesis imperfecta in association with type IB osteogenesis imperfecta (OI) (**a-c**) and type IVB OI (**d**). (**a**) In the irregular, atubular areas of dentin, the collagen fibers occasionally form tightly packed bundles (C), which alternate with loose-textured areas (A) lacking fibrillar collagen. (**b**) Especially the borders of the collagen fiber bundles show abnormally thick hyperfibers (H; also seen in **c**), in which the increase of diameter is accompanied by the loss of a clear cross-striational pattern (arrow). (**c**) Pathological vesicular structures, surrounded by a trilaminar membrane (arrows), are seen in the dentin matrix [electron micrographs illustrating the affected dentin of this patient have been published earlier in Waltimo (1994)]. (**d**) Abnormally thin fibers (arrows) are present among the parallel but loosely arranged collagen fibers. Note also the tendency of either the entire fiber or the ends of the fibers to unravel into fine filaments (arrowheads). Bars = 1 μm (a); 200 nm (b and c); 400 nm (d).

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associated with cross-striated collagen fibers by immuno-TEM (Waltimo *et al.*, 1994). Type VI collagen (found in types I and II DI, but not in normal teeth) seemed, on the other hand, to be located in delicate material, intermingling with the cross-striated collagen fibers (Waltimo *et al.*, 1994). Thus, abnormalities in the constitution of dentin matrix in OI do not appear to be restricted to the genetically defective type I collagen.

Discussion and Conclusions

The particular properties of dentin matrix as a dense connective tissue, forming a framework for the mineral phase, make it a difficult subject to study. The inconsistent data, regarding the constitution and ultrastructure of normal dentin, complicate the interpretation of findings in abnormal dentin. For example, concepts of the presence in dentin of certain collagen types other than type

I, as well as of the contents of the dentinal tubules, differ. In structural analyses of the (aberrant) dentin matrix, the methods used for fixation and demineralization make it difficult to optimize the conditions for simultaneous examination of cells and dentin matrix. For studies of heritable dentin defects, we have thoroughly demineralized the teeth with EDTA to facilitate detailed analyses of the dentin matrix even at the expense of preservation of the cellular structures.

As seen in TEM, the most characteristic feature of heritably abnormal dentin matrix, i.e., the irregularity and sparsity of dentinal tubules, which is thought to reflect a reduced number of adequately functioning odontoblasts (Siar, 1986), is common to types II DD and types I and II DI. This feature has also been shown in light microscopic and SEM studies, which have extended this finding to type III DI as well (Levin et al., 1983). However, TEM has facilitated the detection of more subtle dentinal structures such as vesicles, hyperfibers and unravelled fibers in OI. Also, it has been possible to compare the sizes and the organization patterns of the collagen fibers in the affected teeth. Independent of the type of the defect, the diameter of the collagen fibers tends to be normal in areas showing a tubular structure, occasionally increased in areas where the collagen is densely packed, and usually decreased in loose-textured areas. Formation of thick collagen fiber bundles is characteristic for type II DD (Waltimo et al., 1991), and bundles parallel with the dentinal tubules are also seen in type II DI (Herold, 1972). Teeth from patients with OI may as well contain thick collagen fiber bundles, but instead of being scattered in the collagenous meshwork, they alternate with areas poor in collagen (Waltimo, 1994).

The finding that coarse fibers, resembling the so-called von Korff fibers [thick fibers present in mantle dentin and presumably produced by subodontoblastic cells of the pulp (Shroff and Thomas, 1992)], were present in circumpulpal dentin in type II DI, led Herold (1972) to suggest that mantle dentin-like matrix had been formed continually. The presence of vesicular formations (normally found only in mantle dentin) in circumpulpal dentin of patients with type I DI (Waltimo, 1994, Waltimo et al., 1994), supports this interpretation. On the other hand, dentin in different types of DI closely resembles tertiary or reparative dentin (Kerebel, 1975) which is deposited by pulpal cells differentiated into dentin-forming cells (Lesot et al., 1993). This may happen in normal teeth after the destruction of odontoblasts due to caries, for instance. Reparative dentin has a similarly irregular organic matrix (for review, see Karjalainen, 1984) and calcification front (Levin et al., 1980, 1983), and it also contains matrix vesicles (for review, see Bonucci, 1984) as well as type III collagen

(Karjalainen et al., 1986; Magloire et al., 1988), as does dentin in types I and II DI (Sauk et al., 1980; Lukinmaa, 1988; Waltimo et al., 1994). Furthermore, the lamellar structure of dentin (Aldred, 1992), a zonated pattern of immunostaining for cellular fibronectin (which is not a normal constituent of dentin) described in one patient (Lukinmaa and Vaheri, 1994), and entrapment of cells in the dentin matrix in types I and II DI, suggest that at least in some cases, the original odontoblasts fail to complete dentinogenesis and that other cells, presumably pulpal fibroblasts differentiated into hard tissue-forming cells, continue the formation of dentin (Kerebel, 1975; Waltimo, 1994). This might also explain the deficiency of the dentin-specific phosphoprotein in the affected dentin.

Like von Korff fibers, fibers showing type III collagen reactivity occasionally seem to extend from the pulp to predentin (of intact or carious teeth), and pass the odontoblasts (Becker et al., 1986; Nagata et al., 1992, Lukinmaa et al., 1993; Ohsaki and Nagata, 1994). Despite that odontoblasts appeared to express mRNA for type III collagen during early stages of dentinogenesis (Lukinmaa et al., 1993), dentin matrix may contain products of the dental papilla cells and/or remnants of pre-existing tissue. In a light microscopic and TEM study on developing abnormal teeth of patients with OI, Godfrey (1973) found that odontoblasts, after having formed the normal-appearing mantle dentin, lost their columnar shape and confluent pattern of organization. He suggested that such odontoblasts were incapable to resist involution of pulp tissue into dentin, which hence contained capillaries and collagen fiber bundles of non-odontoblastic origin (Godfrey, 1973). Thus, in the case of DI, the origin of dentin matrix may, in fact, be "dual" (Shroff and Thomas, 1992) in the sense that cells other than the first-generation odontoblasts may contribute to the formation of dentin.

The ultrastructural features of the heritable dentin defects differ to such an extent that their classification into distinct entities is justified, at least until otherwise indicated by further molecular genetic studies. However, there are also many similarities which can be assumed to be secondary in nature, i.e., to result from interactions between the matrix macromolecules. For example, types I and III collagen tend to form mixed fibers (Henkel and Glanville, 1982). Also fibronectin, proteoglycans/glycosaminoglycans, and possibly types V and VI collagen as putative organizers of connective tissue matrix (Holbrook and Byers, 1989) could be affected. Through such molecular interactions, shared by different connective tissues, a single, genetically defective molecule could cause a variety of structural abnormalities. For example, the changes in collagen fibers in abnormal teeth resemble those observed in affected skin. These

include variation in fiber size, formation of hyperfibers/ composite fibers, and presence of uncoiled fibers (Holbrook and Byers, 1989; Waltimo, 1994; Waltimo *et al.*, 1994; Waltimo and Lukinmaa, unpublished observation). The finding that there is a limited repertoire of change in collagen fibrils in the skin (Holbrook and Byers, 1989) also seems to be true for dentin. Thus, the aberrations do not appear to be tissue-specific.

The need for further ultrastructural studies on heritable dentin defects, both classified and thus far unclassifiable, is obvious. While the morphology of the aberrant dentin may be clarified by TEM, more detailed information of the molecular composition of the abnormal dentin matrix may be obtained by immuno-TEM. Particularly in OI, where the manifestations of DI can be so mild as to remain beyond the level of detection of other diagnostic methods, histological and ultrastructural studies are likely to increase the estimated frequency of the dental defect. Furthermore, dental aberrations occur in association with a variety of diseases other than OI (Witkop, 1975; Gorlin *et al.*, 1990), and the possible ultrastructural changes in dentin of those patients provide an unexplored field.

Acknowledgements

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Discussion with Reviewers

H. Lesot: What causes obliteration of the pulp chambers in type I and type II DD as well as in type II DI? In case a single mechanism is involved in all cases, which is not sure, is it due to a deregulation of odontoblast metabolism, to a change in the activity of pulp cells or to more complex processes?

Authors: The mechanism(s) causing obliteration of the pulp chambers not only in type II but also in type I DI, as well as in type II DD (in deciduous teeth), and partially in type I DD, are unknown. The possible explanations, which may even differ in the distinct types of heritable dentin defects, are discussed in this paper. These include abnormal, continual secretory function of the odontoblasts, contribution to the formation of the abnormal dentin matrix by pulpal cells differentiated into hard tissue-forming cells, or both. Because such teeth are usually not obtained until the pulp chamber has been completely obliterated, the precise nature of obliteration is difficult to clarify.

H. Lesot: How is the dentin-enamel junction affected in the different DD and DI?

Authors: In some earlier reports, the dentin-enamel junction was found to be smooth in DI, and this was thought to explain the chipping of the apparently normal enamel. Later, a scalloped junction was, however, observed in some teeth (Sunderland and Smith, 1980). We have also found that the degree of scalloping (which is low even in normal deciduous teeth) varies from tooth to tooth.

H. Lesot: According to what is mentioned in the **Discussion and Conclusions**, could the authors comment on the similarities and differences when comparing dentin(s) formed in type I and II DI with reparative dentin?

Authors: Dentin matrix in both type I and type II DI is similar to that in reparative dentin in terms of the reduced number of dentinal tubules, the irregular tubular pattern, immunoreactivity of type III collagen, as well as the poor mineralization (for review, see Karjalainen, 1984). Information of an irregular calcification front concerns type I DI (Levin *et al.*, 1980) and types II and III DI (Levin *et al.*, 1983). Vesicular structures have been documented in type I DI only (Waltimo, 1994).

H. Lesot: Why are there different manifestations in primary and permanent teeth? What are the subjacent mechanisms?

Authors: The different manifestations in primary and permanent teeth are still a puzzle. So is the absence of DI in the majority of patients with OI. Furthermore, of the permanent teeth those, which develop first, are often most severely affected by both inherited enamel and dentin defects. Hopefully, further studies on normal tooth development will also help us to better understand the abnormal development.

H. Lesot: It is suggested that epithelial cells could primarily be responsible for type I DD. Could the authors comment on this point?

Authors: For decades, the reason for defective root formation in type I DD has been a matter of discussion. Histological findings on the abnormal structure of Hertwig's epithelial root sheath in type I DD, and the consequent suggestion that the defect could reside in the epithelial component (Sauk *et al.*, 1972; Witkop, 1989), were contradictory with the earlier general belief that mesenchymal cells guided tooth development. Later, it was shown that the information was in the epithelium (Mina and Kollar, 1987) in early stages of tooth morphogenesis. Now we know that tooth results from sequential, reciprocal inductive epithelial-mesenchymal interactions. Even currently, the site of the very first signal or tooth-specific information is not known. Even though root formation has not been studied as extensively as crown formation, the mechanism behind type II DD may also well be related to the epithelial-mesenchymal interactions since the level of transition from the normal coronal dentin to the abnormal radicular dentin in permanent teeth affected with type II DD coalignes

with the level of enamel-cementum junction (Ranta *et al.*, 1990, 1993).

H. Lesot: How do you explain the genesis of "branched tubules"? Is this observation confirmed at the ultrastructural level?

Authors: Branched tubules are also seen in normal dentin. The side branches presumably form in the same manner as do the main tubules and result from branching of the odontoblast process. Narrow extensions of odontoblast processes have been shown to fill the side branches, which are thus not artefactual. At the ultrastructural level, the branching can be seen in longitudinal sections, and the side branches appear as small, round openings in transverse sections. The extensive branching of the tubules in DI, which is evident both in light microscopic and ultrastructural examination, is one of the irregularities associated with odontoblasts/dentin matrix in this disease group. Why or how the odontoblasts send side branches is not known.

H. Lesot: The irregularity and sparsity of dentinal tubules was reported "to reflect a reduced number of adequately functioning odontoblasts". What does this mean and do the authors think that this might reflect any common alteration in different diseases?

J.H.M. Wöltgens: I miss findings on odontoblasts or cells of the subodontoblastic layers supporting the hypothesis quoted above. The arguments for this are based on fiber structures only in the absence of cells or non-collagen matrix proteins and are thus indirect. Please comment.

Authors: The statement quoted was made by Siar (1986). It is also in line with our own findings on DI (particularly type I) that, compared to mantle dentin, the abnormal inner dentin often contains a smaller number of dentinal tubules per area. This would mean that part of the odontoblasts fail to produce dentin matrix normally, and thereby, to continue their normal pathway in a pulp-ward direction. The abnormally functioning odontoblasts probably become entrapped in the newly formed defective dentin matrix as also suggested by the presence of cells in dentin (this can be seen in histological sections). We agree that the statement (Siar, 1986) is based on indirect findings and it only concerns teeth affected by DI, not all types of heritable dentin defects. Because these teeth are not obtained at a stage when the dentin is still being formed (for an exception, see Godfrey, 1973), the analysis of the cells is difficult, and the speculations cannot be based on observations other than indirect ones.

O. Johari: Could you please give a reference for the staining methods used?

Authors: The hematoxylin and eosin as well as Schmorl's picric acid and thionin methods have been described, for example, by Stevens (1982) and Page (1982), respectively.

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