Hereditary Defects of Tooth Dentin. Recent Progress on Genetic Aetiology Suggests for Modifications of the Existing Classification System

Summary

Hereditary defects of dentin include dentinogenesis imperfecta (DGI) and dentin dysplasia (DD). They are characterized by abnormal dentin formation. Within the last 32 years, since the first classification system was proposed, significant advances have been made regarding their genetic aetiologies. In the classification system suggested by Shields et al (1973), dentinogenesis imperfecta type I (DGI-I) is associated with some types of osteogenesis imperfecta (OI), which is caused nearly in all cases by mutations of the genes encoding type I collagen (Col1A1 and Col1A2 genes). However, a more specific relationship between the type of OI, the genetic defect and the dental involvement can not been established. As far as isolated dentin defects are concerned, 10 mutations all occurring in different sites of the DSPP gene have been described to cause DGI-II, DGI-III and DD-II. No information about the gene defects in DD-I is currently available. Plenty of new evidence suggests that the existing classification system should be revised at least as far as the types of dentinogenesis imperfecta II and III are concerned.

Keywords: DSPP Gene; Mutations; Dentin Dysplasia; Dentinogenesis Imperfecta

Introduction

2 main groups, Dentinogenesis imperfecta (DGI) and Dentin dysplasia (DD), have been identified under the term “dentin genetic diseases” by using clinical, radiographic and histopathologic features. Both exhibit an autosomal dominant pattern of inheritance.

Dentinogenesis imperfecta (DGI) is the most prevalent human genetic disease affecting dentin formation with an estimated incidence between 1:6000 to 1:8000 in the United States. Association of dentinogenesis imperfecta with autosomal recessive disorders is extremely rare. DGI is further divided into 3 subgroups (types I-III). The dental defects associated with some forms of osteogenesis imperfecta (OI) has been defined as DGI type I (DGI-I). Osteogenesis imperfecta also known as “brittle bone disease” is an inherited clinical syndrome marked by skeletal fragility and other associated abnormalities, including dentinogenesis imperfecta. According to the most frequently used classification, 4 main types of OI are recognized (Tab. 1), the last type (IV) including all individuals who are not clearly part of the first 3 types. From this heterogeneous group, 3 separate clinical entities on the basis of distinct clinical and bone histological features have been identified more recently and termed types V, VI and VII. The incidence of DGI is highest in OI types III and IV and less in type I, while there are no reports associating DGI with types V, VI, and VII.

DGI types II and III (DGI-II and DGI-III) occur as isolated traits. DGI-II, also called “hereditary opalescent dentin”, presents almost complete penetration, a high expressivity and a low frequency of de novo
mutations. DGI-III was first described in an isolated triracial population in southern Maryland known as the “Brandywine isolate”.

In all DGI types the teeth have a variable blue-grey to yellow brown discoloration that appears opalescent due to the defective, abnormally coloured dentin, shining through the translucent enamel. Enamel, although normal in structure, due to defective dentin frequently fractures from the teeth leading to rapid wear and attrition of the crowns. Radiographically, affected teeth show variable expression of bulbous crowns, cervical constrictions and short roots. An important difference between the DGI types is that the teeth with DGI-III, in comparison with the other 2 types, radiographically show abnormally large pulp chambers and no pulpal obliteration. Dentine in all types of DGI appears histologically similar, having reduced numbers of tubules, irregular tubular morphology, immunoreactivity for type III collagen and poor mineralization.

Dentin dysplasia (DD) is a less frequent disease and it is subclassified into 2 types. In type I (DD-I) the teeth have a slight amber discoloration and are often mal-aligned. They appear radiographically short conical shaped roots with apical constrictions and pre-eruptive pulpal obliterations, which results in a crescent shaped pulp chamber. There are usually numerous periapical radiolucencies in DD-I that have essential diagnostic appearance. The description of DD-II as given by Shields et al. is consistent with most other reports, but that of DD-I is probably too limited and the precise nature of the defect in DD-I has not been determined yet.

A number of systemic connective tissue disorders that can cause changes in dentin structure exist, such as OI, Ehlers-Danlos and Goldblatt syndrome. One of the problems of the currently used classification system is that only OI is recognized. Another problem is that the problems of the currently used classification system, with respect to the classification system.

**Genes and Related Proteins**

**Candidates Dentin Diseases**

Tooth development is a highly organized process involving complex interactions among a number of genes. Disordered gene expression at the early stages of this process can arrest it. In contrast, genetic defects manifested at later stages (crown and root formation) and more specifically during matrix deposition of dentin are believed to result in malformations that occur exclusively within the tissue. Consequently, candidate gene approaches to characterize the specific aetiologies of dentinogenesis imperfecta and dentin dysplasia focus on mutational analyses of the genes encoding dentin matrix proteins.

Dentin is a mineralized tissue whose composition and mode of formation are relatively similar to those of bone. Bio-mineralization of dentin extra-cellular matrix requires complex interactions among several collagenous and non-collagenous molecules, secreted by the odontoblasts. The bulk of the organic matrix of dentin (85-90%) consists of collagen. Most of the collagen is type I with minor amounts of type V and type I trimer. Collagen fibrillogenesis is a precisely regulated process that ultimately defines the overall extra-cellular matrix (ECM) assembly and function, providing the structural scaffold necessary for mineralization.

The non-collagenous molecules can be subdivided into several broad categories: phosphoproteins, non-phosphorylated matrix proteins, proteoglycans, growth factors, amelogenin 5-7 kDa, growth factors, metalloproteinases, serum-derived proteins and phospholipids. Although the exact mechanisms in dentinogenesis are not yet elucidated, the experiments so far indicate that the non-collagenous proteins (NCPs) have a central role in orchestrating this process. The phosphoproteins are of particular interest, as they appear to promote actively the mineralization of collagen fibres and crystal growth within pre-dentin when this tissue is converted to dentin. One category of the NCPs is termed the SIBLING (Small Integrin-Binding LIgand, N-linked Glycoprotein) family, which includes bone sialoprotein (BSP), osteopontin or secreted phosphoprotein-1 (OPN or SPP1), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSP), and consequently DPP and DSP, matrix extra-cellular phosphoglycoprotein (MEPE/OF45) and others. All members share common features at the protein and gene level.

The genes coding for these proteins have been mapped to human chromosome 4q21 within a gene cluster, in the overlapping region of the DD-II, DGI-
II and DGI-III critical loci\(^{38-41}\) (Fig. 1), and therefore they were considered candidate genes for dentin diseases. Up to date, strong evidence for a causative role exists only for the DSPP gene and studies have shown the association of mutations in this gene with DGI and DD-II. DMP1 is located between DSPP and BSP (IBSP) gene (Fig. 1) and was appointed as candidate gene for DD-II, DGI-II and DGI-III. However, mutational analyses up to date exclude this gene from a causative role in the pathogenesis of DGI-II and DGI-III, at least within the families studied\(^{32-33}\). BSP and OPN were also strong candidates for DGI-II since linkage analysis using 2 large families with DGI-II demonstrated no recombination events with this disease\(^{33}\). However, mutation search in both OPN and BSP in individuals with DGI-II yielded negative results\(^{34,44}\). MEPE/OF45 is located between BSP and OPN and thus was indicated as another possible candidate gene for dentin diseases\(^{37}\). To date, no mutational analysis of MEPE/OF45 has been reported to support this hypothesis.

**Figure 1.** High resolution mapping of the overlapping region (between markers SSP1 and D4S2691) of the DD-II, DGI-II and DGI-III critical loci in human chromosome 4(4q21). Candidate genes for dentinogenesis imperfecta and dentin dysplasia are shown in bold.
Dentin Diseases and Genetic Background

Mutations in the DSPP Gene; Defective Gene Expression and Its Association with Isolated Dentin Defects

The DSPP gene in all species studied to date\textsuperscript{45-48}, is organized into 5 exons and 4 introns. The human DSPP contains an open reading frame of 3759 bp encoding for a polypeptide with 1253 deduced amino acid residues. The 3' portion of exon 2 together with exon 3, the 4 and 5' portion of exon 5 encodes for DSP and DSP-DPP linker region (283-462 amino acids), while the remainder of exon 5 encodes for DPP\textsuperscript{46-48} (Fig. 2).

Following the cloning and characterization of the human DSPP gene\textsuperscript{46}, mutational analyses, conducted in families with inherited dentin defects, have identified 10 different disease-causing mutations. All these mutations are inherited in a dominant way and can be divided into 4 categories: missense, nonsense, splice-site and compound mutations (Fig. 2). During all these studies, the researchers had to face a common problem related to the fact that the DSPP gene has a narrow pattern of expression, and thus there were no tissues that could be easily biopsied to gain DSPP mRNA from the affected individuals. Consequently, the sequence of the mutated DSPP transcripts could not be determined.

![Figure 2. Human DSPP gene its encoding protein and reported mutations. The intron-exon structure of the human DSPP gene (Gu et al, 2000). The numbered cylinders represent the exons, the line the introns. Translation of the signal sequence initiates in exon 2 (view shape ▲ in diagram) and translation stop codon is at exon 5 (view shape ▼ in diagram). Vertical arrows show the location of the mutations, while the branched boxes include the genomic DNA (upper row) and the mutated protein (lower row). The associated dentin defect with each mutation as well as the references where each mutation is described, are depicted below the gene representation.](image)

**Abbreviations:** SP: signal peptide, DSP: dentine sialoprotein, DPP: dentine phosphoprotein, DSPP: dentine sialophosphoprotein
The first mutation in the DSPP gene was described by Zhang et al\textsuperscript{49} in a Chinese family. It was a nonsense mutation at nucleotide 3658 (g.1272C→T), which introduced a premature stop codon in exon 3. This premature stop codon was predicted to result in the absence of DPP and a greatly shortened DSP - only 29 amino acids (Fig. 2). The same year, Xiao et al\textsuperscript{50} reported 3 more disease-specific mutations. Their study included 3 Chinese families diagnosed with DGI-II. Furthermore, the affected individuals in 2 of them additionally presented progressive sensory-neural high-frequency hearing loss (Fig. 2). In the first family (the one without hearing loss) they detected a G→A transition at the donor-splicing site of intron 3 (g.1275G→A), a mutation predicted to result in the skipping of exon 3, which encodes part of DSP protein. In the second family, all the affected members carried a missense mutation at codon 17 (g.1474A→T) predicted to result in a substitution of Pro by Thr, while in the third family all affected members carried a G→T transversion at codon 18 (g.1191G→T) predicted to result in a substitution of Val by Phe (splice site mutation). These 2 mutations were considered responsible for the DGI-II phenotype as well as for the progressive high-frequency hearing loss present in the affected family members. At the protein level, it was suggested that these 2 mutations might have interfered with the cleavage of the signal peptide. Given that these 2 mutations occurred in the DSP portion of DSPP, they concluded that they predominantly affected the function of the DSP protein. However, one cannot exclude that changes in the expression pattern of DPP and the localization of DPP protein might have occurred as well.

Rajpar et al\textsuperscript{51} reported a missense mutation at nucleotide 16 (g.16T→G) that resulted in the substitution of the amino acid Tyr by Asp within the hydrophobic core of the DSPP signal peptide domain (Fig. 2). They suggested that this mutation might have interfered with the translocation of DSPP to the endoplasmic reticulum during protein translation and impeded the secretion of the protein, so that roughly half of the normal amount of the protein was present in the dentin extra-cellular matrix. This mutation caused a DD-II phenotype where the permanent protein was present in the dentin extra-cellular matrix. This is the third mutation in the DSPP gene that has been associated with DGI-II. In contrast, they found that a rare compound mutation in the DSP portion of the DSPP gene was responsible for the observed phenotype in the affected family members. The first alteration was a 36 bp deletion (3599_3634del GT GAC AGC AGT GAC AGC GAC AGC AGT GAC AGC A) while the second alteration was a 18 bp insertion (3715_3716ins GC GAT AGC AGT GAC AGC A) (Fig. 2). This compound mutation resulted in an in-frame truncation of the DPP domain by only 6 amino acids near the highly conserved carboxyl terminus. These mutations altered the length of the repetitive segments (DSS) within the DPP domain, affecting the overall function of the DPP protein. The authors suggested that the mechanism of this mutation may be similar to that observed in neurological diseases caused by trinucleotide repeats mutations\textsuperscript{53,55}. A more detailed knowledge on DSPP’s role in bone formation is needed in order to understand how ear deformities can result from mutations in the DSPP gene and how they may contribute to the pathology of progressive high frequency hearing loss.

Kim et al\textsuperscript{56} investigated the possible roles of DMP1 and DSPP in the pathogenesis of DGI-III, in a family located in the Brandywine region of southern Maryland and previously diagnosed with DGI-III. Their study indicated that no mutations within DMP1 were associated with DGI-III. In contrast, they found that a rare compound mutation in the DPP portion of the DSPP gene was responsible for the observed phenotype in the affected family members. The first alteration was a 36 bp deletion (3599_3634del GT GAC AGC AGT GAC AGC GAC AGC AGT GAC AGC A) while the second alteration was a 18 bp insertion (3715_3716ins GC GAT AGC AGT GAC AGC A) (Fig. 2). This compound mutation resulted in an in-frame truncation of the DPP domain by only 6 amino acids near the highly conserved carboxyl terminus. These mutations altered the length of the repetitive segments (DSS) within the DPP domain, affecting the overall function of the DPP protein. The authors suggested that the mechanism of this mutation may be similar to that observed in neurological diseases caused by trinucleotide repeats mutations\textsuperscript{53,55}. A more detailed knowledge on DSPP’s role in bone formation is needed in order to understand how ear deformities can result from mutations in the DSPP gene and how they may contribute to the pathology of progressive high frequency hearing loss.

Kim et al\textsuperscript{56} in a study that included a Korean and a Caucasian family identified a G→T substitution at the first nucleotide of exon 3 of the DSPP gene. The same mutation was previously described by Xiao et al\textsuperscript{50} in a Chinese family. The clinical and radiographic features of these 2 families included the classic phenotypes associated with...
both DGI-II and DGI-III. At the protein level, the authors suggested that the mutation might impede the secretion of the protein or interfere with the cleavage of the signal peptide. Alternatively, this substitution might alter or destroy the function of the secreted protein by affecting the tertiary and or its quaternary structure.

Song et al in the study which included members from 2 Chinese families diagnosed with DGI-II identified 2 previously described mutations causing the disease - a nonsense mutation (c.133 C→T) in family 1 and a missense mutation (c.52G→T) in family 2. The affected members of the families in this study showed a remarkably different phenotype, in comparison with the families reported in other studies having the same mutations. It was therefore suggested that the c.133C→T and c.52G→T could be 2 mutation hotspots, causative for different clinical phenotypes in multiple unrelated DGI families. More recently, Zhang et al studying a 4-generation Chinese family diagnosed with DGI-II identified a novel missense mutation in exon 2 (c.49C→T) of the DSPP gene, that resulted in the substitution of the Pro17 residue by Ser. The mutation was identified in all the affected individuals, but not in normal family members and 100 controls.

The identification of several mutations in the DSPP gene in families diagnosed with DGI and DD-II does not necessarily pre-exclude that other genes are also involved in the aetiology of dentin diseases. Namely, in a recent study, it was shown that in 3 out of 4 families with DGI-II, not any mutation was identified in the DSPP gene. This suggests that at least in some cases of DGI-II the responsible defective gene is other than the DSPP, or these cases are caused by mutations located in untranslated regions or introns of the DSPP gene and that could affect DSPP gene function.

### Spectrum of Dental Aberrations in Osteogenesis Imperfecta

Biochemical and molecular genetic studies have shown that the vast majority of individuals (90%) affected with OI types I-IV, have mutations in either the Col1A1 or Col1A2 genes that encode the pro-α1 and pro-α2 chains of type I collagen. It is believed that DGI-I, when present in patients with OI (Types I-IV), most likely reflects the fact that type I collagen is the main organic component in both dentin and bone. Numerous studies have shown that there is a clear relationship between the degree of dentin dysplasia and the type and form of OI. Thus, teeth from patients with OI type III have a higher degree of dysplasia than teeth from patients with other types, and an increasing severity of the disease is associated with an increasing degree of dysplasia. This indicates a strong correlation between the genotype in OI and the presence or absence of DGI. Recently, Pallos et al in a Brazilian family with OI type IV, have identified a Gly559Cys mutation in exon 32 of the Col1A1 gene present in all DGI-affected members but not in the individuals without DGI-I. This finding supports the notion that DGI-I might be associated with specific mutations in the Col1A1 and Col1A2 genes.

In the case of OI, the resulting phenotype can vary from very mild to lethal depending on which of the 2 α chains is affected, the position in the triple helix at which the substitution arises and which is the substituted amino acid. Although it is not known how odontoblasts process mutated gene products or how much mutated protein is secreted and whether this is incorporated into the organic matrix, it has been proposed that α-chain stoichiometry does not affect the teeth and aberrations in the α2(I) chain are more important for the development of DI than those in the α1(I) chain, which may be substantiated by the more frequent involvement of the α2(I) than the α1(I) chain in DI.

### Table 1. Osteogenesis imperfecta types, genetic aetiology and dental aberrations

<table>
<thead>
<tr>
<th>Osteogenesis imperfecta type</th>
<th>Association with dentinogenesis imperfecta</th>
<th>Mutations in collagen genes (Col1A1 and Col1A2)*</th>
<th>References</th>
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<tr>
<td>I</td>
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<td>Sillence   (1988)</td>
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<td>II</td>
<td>-</td>
<td>+</td>
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<td>III</td>
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<td>V</td>
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<td>No evidence for collagen type I abnormality</td>
<td>Glorieux et al. (2000)</td>
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<tr>
<td>VI</td>
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<td>No evidence for collagen type I abnormality</td>
<td>Glorieux et al. (2002)</td>
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<tr>
<td>VII</td>
<td>-</td>
<td>Unknown genetic defect.</td>
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<td></td>
<td></td>
<td>No evidence for collagen type I abnormality</td>
<td>Glorieux et al. (2002)</td>
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* Col1A1: Collagen I alpha1 gene Col1A2: Collagen I alpha2 gene
The genetic defect underlying OI types V-VII remains to be elucidated as it does not appear to be associated with collagen type I (Tab. 1). A recent publication describing a deletion in the gene sphingomyelin phosphodiesterase 3 (Smpd3) that resulted in osteogenesis and dentinogenesis in mice indicates that sphingomyelinasases are deeply involved in bone and dentin mineralization and, on the other hand, provides evidence that different etiopathological mechanisms are involved in non-collagenous OI55.

Discussion

Our knowledge on the aetiology of isolated dentin defects has been promoted after the description of mutations in the DSPP causing DD-II, DGI-II and DGI-III. So far, no disease-causing mutations outside of the DSPP have been identified.

Overall, the data from mutational studies strongly suggest that the classification system should be revised as phenotypic differences that have been taken into account for this purpose5 do not appear to have a correlation with the differences in the genetic background. More specifically, the fact that firstly DD-II, DGI-II and DGI-III are caused by mutations in the same gene, secondly that DD-II overlaps the critical regions for DGI-II and DGI-III54, thirdly they appear phenotypic similarities, all the above indicate that DD-II, DGI-II and DGI-III are allelic and represent a spectrum rather than distinct entities52. This is further supported by the recent study of Kim et al56, in which it was shown that a single mutation (g.1191G→T) is underlying both DGI-II and DGI-III phenotypes. Moreover, Song et al58 found that 2 mutations previously described and causative for a DGI-II phenotype in the families they studied, were causative of a phenotype close to that of DGI-III. It was therefore suggested, that these 2 types should be recognized as phenotypic variations of a single disease, with differences in expressivity and severity and the term “Hereditary opalescent dentin” should be used to describe both the DGI-II and DGI-III types.

With respect to the severity of the phenotype, there is evidence suggesting that an important determinant of it is the locus of mutation in the DSPP gene, since this affects the amount of functional DSP and DPP proteins present in the dentin matrix. Thus, mutations that cause major variations in the amount of functional DSP and DPP (e.g. the p.Y16D mutation) will result in more severe phenotypes than mutations that cause subtle changes in the amount of one or both of them. The study of Malmgren et al52 is representative of this assumption. The more severe phenotype in the members of family B was attributed to the great reduction in the amount of DSP and DPP caused by the mutation in exon 2. The phenotype of this family was similar to the DD-II phenotype reported by Rajpar et al51. There is a possibility, that mutations interfering with function of the signal peptide or its cleavage result in a DD-II or similar phenotype, whereas mutations in the central region or the carboxyl terminus result in a DGI-II or a DGI-III phenotype. However, relatively few mutations have been published so far and the establishment of genotype-phenotype correlations remains a difficult task.

Conclusions and Future Directions

Mutations in the DSPP have been shown to cause DD-II and “hereditary opalescent dentin”. However, it is possible that genes other than DSPP might be involved in the aetiology of some cases. DMP1 and MEPE/OF45 remain candidate genes for dentin diseases, but their involvement in the pathogenesis of these diseases has yet to be proven. The role of MEPE/OF45 in dentinogenesis and consequently in dentinogenesis imperfecta is far from clear. Towards this direction, the study of the tooth phenotype in MEPE null-mice will help establishing possible relationships (if any) between this protein and bio-mineralization of dentin.

The genetic defect behind DD-I is currently unknown, but it is believed to be different from that of DD-II. Future genetic research might also determine whether DD-I has various expressions or needs sub-classification. DGI-I, as originally described, is associated with some forms of OI (III, IV and I types). Currently, a more definite relation between mutations in the collagen genes and DGI-I cannot be established, something that would substantiate the higher incidence of DGI-I in certain OI types. As more mutations regarding OI and DGI-I are described, the correlation between clinical and molecular data may be better understood. A future goal could be the development of a classification system for dentin genetic diseases according to their genetic background.

References


Correspondence and request for offprints to:
Lyroudia Kleoniki
23, Papafli Str.
54638 Thessaloniki, Greece
E-mail: lyroudia@zeus.csd.auth.gr