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Phenotypic Variation in Dentinogenesis Imperfecta/Dentin Dysplasia Linked to 4q21

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Abstract

Dentinogenesis imperfecta (DGI) and dentin dysplasia (DD) are allelic disorders that primarily affect the formation of tooth dentin. Both conditions are autosomal-dominant and can be caused by mutations in the dentin sialophospho-protein gene (*DSPP*, 4q21.3). We recruited 23 members of a four-generation kindred, including ten persons with dentin defects, and tested the hypothesis that these defects are linked to *DSPP*. The primary dentition showed amber discoloration, pulp obliteration, and severe attrition. The secondary dentition showed either pulp obliteration with bulbous crowns and gray discoloration or thistle-tube pulp configurations, normal crowns, and mild gray discoloration. Haplotype analyses showed no recombination between three 4q21-q24 markers and the disease locus. Mutational analyses identified no coding or intron junction sequence variations associated with affection status in *DMPI*, *MEPE*, or the DSP portion of *DSPP*. The defects in the permanent dentition were typically mild and consistent with a diagnosis of DD-II, but some dental features associated with DGI-II were also present. We conclude that DD-II and DGI-II are milder and more severe forms, respectively, of the same disease.

Keywords

dentin; dentin sialophosphoprotein; *DSPP*; dentinogenesis imperfecta; dentin dysplasia

INTRODUCTION

The clinical classification system that is often used to categorize inherited defects of tooth dentin was first proposed to make a distinction for a pattern of dentin defects, designated dentin dysplasia type II (DD-II), that featured “amber, translucent colouration and total pulpal obliteration in all primary teeth” and permanent teeth with “thistle-tube pulp configuration with ubiquitous pulp stones and normal colouration” (Shields *et al.*, 1973). This classification system, now in use for over 30 years, divides inherited dentin defects into two disease groups: dentinogenesis imperfecta (DGI, types I–III) and dentin dysplasia (DD, types I and II). This system, however, was never intended “to be the final work on the etiologic classification of dentin diseases, about which little is known of the basic molecular defect” (Bixler, 1976).

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As more is known about the etiology of non-syndromic heritable dentin defects, the more they appear to be manifestations of a single disease. The distinctions used to compartmentalize the spectrum of phenotypic variations observed in kindreds with inherited defects of dentin into dentinogenesis imperfecta and dentin dysplasia may reflect differences in severity rather than in the presence, absence, or grouping of defined pathological features. DGI-I is the dental phenotype associated with osteogenesis imperfecta (OI) (Pallos *et al.*, 2001). People with this disorder are best classified as having osteogenesis imperfecta with DGI (OI/DGI) (O'Connell and Marini, 1999). Defects in the human dentin sialophosphoprotein (DSPP) gene can cause DGI-II (Xiao *et al.*, 2001; Zhang *et al.*, 2001; Kim *et al.*, 2004, 2005), DGI-III (Dong *et al.*, 2005; Kim *et al.*, 2005), and DD-II (Rajpar *et al.*, 2002). No mutation in any gene besides *DSPP* has been shown to cause non-syndromic heritable dentin defects. While it is premature to rule out a role for other genes, the hypothesis that only *DSPP* is involved in the etiology of DGI-II, DGI-III, and DD-II is consistent with the assertion that "heritable dentine defects can be viewed as a continuum" (Shields *et al.*, 1973).

We have characterized a four-generation kindred displaying inherited dentin defects associated with both DD-II and DGI-II (Witkop, 1975; Bixler, 1976). While the *kinds* of defects vary within our kindred, the *severity* of the defects is generally mild and most consistent with a diagnosis of dentin dysplasia type II. Pulp chamber obliteration occurs after eruption, and the dental phenotype does not lead to accelerated attrition of the permanent teeth. In this study, we describe the variations in dental phenotypes among affected members of this kindred, and investigate the genetic etiology by performing linkage and mutation analyses in the critical region of chromosome 4q21-q24.

MATERIALS & METHODS

Enrollment of Human Subjects

The study protocol and patient consents were reviewed and approved by the Institution Review Boards at the University of Michigan. Twenty-three members of a four-generation kindred consented to participate in the study and contributed samples for genomic DNA analyses. Medical and dental histories and oral photographs were obtained for 17 family members (I-2; II-5, 6, 7, 8, 9, 11; III-4, 6, 7, 8, 9, 10, 11, 12; IV-1, 2). Dental radiographs were obtained from 14 of these members (excepting II-8, IV-1, IV-2). Partial previous dental records were obtained for five family members (II-8, II-9, III-9, III-10, III-11).

Primer Design, Polymerase Chain-reaction (PCR), and DNA Sequence Analyses

High-molecular-weight genomic DNA was isolated with use of the QIAamp DNA Blood Maxi Kit and protocol (Qiagen Inc., Valencia, CA, USA). Fifty nanograms of genomic DNA from affected individuals were amplified by Platinum Taq High Fidelity (Invitrogen, Carlsbad, CA, USA). PCR amplification products were purified by QIAquick PCR Purification Kit and protocol (Invitrogen). DNA sequencing was performed on an ABI Model 3700 DNA sequencer (Applied Biosystems, Foster City, CA, USA) at the University of Michigan DNA Sequencing Core Facility (<http://seqcore.brcf.med.umich.edu/doc/dnaseq/intro.html>).

Oligonucleotide primers for polymerase chain-reaction (PCR) were designed with Primer3 on the Web (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) (Rozen and Skaletsky, 2000). The strategy was to generate PCR amplification products that would allow for the DNA sequencing of each coding exon and at least 50 bp of adjoining intron. The PCR conditions for the amplification of the coding regions of *DSPP*, *DMP1*, and *MEPE*, and the sequences of the PCR and DNA sequencing primers, are provided in the Table. The PCR primers were also used to prime the DNA sequencing reactions.

We accomplished DNA sequence analysis of the DSPP 5' regulatory region by characterizing 3 overlapping PCR amplification products. The amplimers were 648, 667, and 640 bp in length and were amplified with the following primer pairs, respectively: PrF1 CTGCTTTAGTGACATCCACAG and PrR1 GGAAATTTGCCTTGAAGCTG; PrF2 GCCCTTAATCTGCCCAAGT and PrR2 CGTTGCC TGAGGGATCTTTA; and PrF3 GCACCTTT GGACATCTTGCT and PrR3 CCACTGTCCTGG ACTTTTGC.

Haplotype Analyses

Six polymorphic markers covering the chromosome 4q21-q24 region, inclusive of the DSPP (89cM), were used for haplotype analysis: D4S1592 (72.71cM), D4S392 (80.38cM), D4S2964 (88.45cM), D4S1534 (93.19cM), D4S414 (100.16cM), and D4S1572 (107.52cM) (Broman *et al.*, 1998). The primers were supplied and analysis was carried out by the University of Michigan DNA Sequencing Core Facility. We calculated LOD scores to analyze statistical significance of the linkage of each marker to the disease locus using the MLINK (Curtis and Sham, 1995).

RESULTS

Members of a Caucasian family with inherited defects of dentin were examined at a University of Michigan dental clinic. The family agreed to participate in this study, and DNA samples were obtained from 23 members of the kindred. There was no history of any unusual bone brittleness or unexplained hearing loss in the family. Affection status (the presence of inherited dentin defects) was ascertained by oral examination and analysis of dental radiographs.

The pedigree of the kindred going back 4 generations is shown in Fig. 1. The pattern of inheritance is autosomal-dominant. Although the dental phenotype was relatively mild, there was complete penetrance. A high number of restored or missing teeth in older affected members of the kindred limited our ability to determine their dental phenotypes. Individual I-2 (age 78 yrs) was edentulous and reported that her mother, now deceased, was also affected by the dental disease. Individual II-5 (age 53 yrs) had only 6 natural teeth remaining. Individual II-9 (age 49 yrs) was missing 3 maxillary and 2 mandibular teeth and all of her 3rd molars. She had crowns, bridges, or very large fillings covering all of the remaining maxillary teeth. Individual II-11 (age 43 yrs) retained all of his natural teeth. His dentition was mildly discolored (yellow), and showed significant abrasive wear in the anterior teeth, particularly the maxillary central incisors (not shown).

In generation III, several classic features of inherited dentin defects were evident. Individual III-4 (age 30 yrs) had crowns of normal shape with a mildly gray coloration in some teeth (Fig. 1). Her panorex showed that the pulp chambers of the maxillary anterior teeth were obliterated, while the bicuspid had pulp chambers and root canals that were "thistle tube" in shape. In contrast, individual III-9 (age 23 yrs) had bulbous crowns and obliterated pulp chambers (Fig. 1). Her teeth were more severely discolored gray, with veneers covering all of the maxillary anterior teeth. Bitewing radiographs of her primary teeth showed the effects of accelerated attrition (Fig. 1).

Oral photographs of the two siblings in generation IV show the contrast between the normal primary teeth of individual IV-1 (age 3 yrs) and the affected primary teeth of individual IV-2 (age 2 yrs), which were amber in color and translucent. Although these were the only individuals with primary teeth that were examined, several family members recalled having primary teeth that resembled "popcorn kernels", that wore down to the level of the gingiva.

DNA samples were analyzed from 10 affected and 13 unaffected family members (N = 23). Haplotype analysis of these samples identified three markers, D4S1534, D4S1572, and

D4S414, with statistically significant (> 3) LOD scores (Fig. 2). This haplotype analysis shows the allelic combinations (as indicated by the presence of specific polymorphic markers) that were transmitted from parent to offspring in this kindred. Note that the affected great grandparent (I-2) had allele 4 for marker D4S1534, allele 6 for marker D4S0414, and allele 2 for marker D4S1572. These same alleles were also present in the nine affected offspring in generations II-IV. This indicates that no crossover events (recombination frequency, $\theta = 0$) were observed between the disease locus and these three markers in the 10 affected persons tested. Mutational analyses were performed on genomic DNA from affected individual III-9. No sequence variations were identified in the splice junctions or within the first 4 exons or 5' part of *DSPP* or in the coding exons and adjoining introns of *DMP1* and *MEPE*. Nor were any disease-associated sequence variations detected in the *DSPP* 5' transcriptional regulatory region.

DISCUSSION

In our kindred affected with a mild form of non-syndromic inherited dentin defects, the primary dentition was amber and translucent, and showed an accelerated rate of attrition. The primary teeth were more severely affected than the permanent teeth. These features of the primary teeth, however, are common to all forms of inherited dentin defects and do not help make a more specific diagnosis. The presence of discoloration in both dentitions has been used to draw a distinction between dentin dysplasia and dentinogenesis imperfecta (Bixler, 1976), but the discoloration of the permanent dentition, while a consistent feature of DGI-II, has also been reported in patients with DD-II (Ranta *et al.*, 1990; Salvolini *et al.*, 1999). Several of our subjects (II-9, II-11, III-4) showed thistle-tube-shaped pulp chambers that are a classic feature of DD-II. Another subject (III-9) showed gray-colored anterior crowns having an ovoid or bulbous shape, with obliterated pulp chambers. Some posterior teeth had bulbous crowns with tapering roots. Bulbous crowns, tapered roots, and obliterated pulp chambers are more associated with DGI-II, but even in this subject the dental phenotype was not severe enough to sustain a diagnosis of DGI-II. The radiographic density of the dentin was within normal limits, as was the rate of attrition of the permanent teeth.

A genetic region on the long arm of chromosome 4 that includes *DSPP* has been linked to both DGI-II (Crosby *et al.*, 1995) and DD-II (Dean *et al.*, 1997). One *DSPP* mutation (p.Y6D) has been shown to cause DD-II (Rajpar *et al.*, 2002). Five *DSPP* coding mutations (p.A15V, p.P17T, p.V18F, p.Q45X, p.R68W) and two splice junction mutations (IVS2-3C and IVS3+1) have been shown to cause DGI-II (Xiao *et al.*, 2001; Zhang *et al.*, 2001; Kim *et al.*, 2004, 2005; Malmgren *et al.*, 2004). *DSPP* is a chimeric protein containing three structural domains. Dentin sialoprotein (DSP) is a proteoglycan at the N-terminus (Ritchie *et al.*, 1994; Yamakoshi *et al.*, 2005a); dentin glycoprotein (DGP) is in the middle (Yamakoshi *et al.*, 2005b), and dentin phosphoprotein (DPP) is a highly phosphorylated protein at the C-terminus (Ritchie and Wang, 1996; MacDougall *et al.*, 1997). The *DSPP* mutation (p.Y6D) that generated the mildest phenotype in the permanent dentition (DD-II) involved a single amino acid substitution in the *DSPP* signal peptide. This mutation likely reduced the amount of secreted *DSPP* protein by at most 50% and maybe much less, but the secreted proteins (DSP, DGP, and DPP) were entirely normal. The five *DSPP* coding mutations causing DGI-II were all in the DSP region. Four of these mutations caused single amino acid substitutions that would change the DSP primary structure, suggesting that this proteoglycan has a critical function in dentin formation. The fifth mutation created a translational stop codon that could have caused a small amount of truncated DSP to be secreted, but the main effect would be degradation of *DSPP* mRNA transcripts from the mutant allele. A similar degradation of transcripts was the expected result of the two splice junction mutations. Thus, the major predicted consequence of several of the *DSPP* mutations causing DGI-II was haploinsufficiency, or a reduction by half of the amount of *DSPP* secreted, while DD-II might be expected to have caused a reduction of less than half.

The clinical evaluation of our kindred with DD-II shows that this phenotype overlaps that of DGI-II, and that all of the defining criteria to make the distinction between DD-II and DGI-II are based upon severity. The disease locus in our kindred lies in the 4q21-q24 region, which includes the *DSPP* gene. We were not able to analyze the DPP coding region, because its extreme redundancy precludes PCR mutational analyses. Furthermore, the wild-type sequence of the human DPP coding region is uncertain. At the time of this writing, the *DSPP* exon 5 genomic sequence (NC_000004) is 144 bp longer than the corresponding region of the *DSPP* cDNA reference sequence (NM_014208). The 144-bp difference is spread over an extensive region and requires 11 length adjustments varying between 9 and 63 dashes to maintain the alignment. Even with the length adjustments, there are 18 nucleotide mismatches. Our mutational analyses were able to exclude coding and splice junction mutations outside of the DPP region of *DSPP*, and the *DMP1* and *MEPE* genes, which had previously been proposed as candidate genes (MacDougall *et al.*, 1996, 2002). Analyses of known *DSPP* mutations indicate that when DSPP secretion is reduced by a little, DD-II is the resulting phenotype. When DSPP secretion is reduced by half, DGI-II is the phenotype. We conclude that the genetic and clinical evaluation of kindreds with inherited dentin defects are most consistent with the interpretation that type II dentin dysplasia and dentinogenesis imperfecta are the same disease, differing mainly in the severity of the underlying genetic defect and resulting clinical phenotype.

Our findings support the need for a better understanding of the genetic etiologies of non-syndromic heritable dentin defects, so that the goal of instituting a gene-based classification system can be realized (Dean *et al.*, 1997). A gene-based system would group all of the DD-II, DGI-II, and DGI-III cases that are associated with *DSPP* defects into a single category, and then make distinctions based upon severity. According to the same designations as are currently in use, the DD-II phenotype would be the least severe, DGI-II intermediate, and the DGI-III phenotype the most severe form of the disease.

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Figure 1.

Pedigree and dental/radiographic features of the phenotype. The four-generation pedigree includes 27 persons (**top**). The 23 people enrolled in the study are indicated by an asterisk. Oral photographs are shown from three affected individuals (III-4, III-9, IV-2) and one unaffected (IV-1). Radiographs for III-4 and III-9 are shown below their oral photos. Individual III-4 (age 30 yrs) has a mild gray discoloration to her teeth. Her panorex shows thistle-tube pulp chambers. Individual III-9 (age 23 yrs) has more severe discoloration, which is still evident in her lower anterior teeth. All of the maxillary anterior teeth were veneered for esthetic reasons. Bitewing radiographs of the primary teeth, taken at age 5 yrs, show pulpal obliteration and accelerated attrition. An anterior periapical survey, taken at age 17 yrs, shows bulbous crowns and pulpal obliteration. The primary teeth of affected individual IV-2 at age 2 yrs show amber discoloration and coronal translucency, in contrast to the normal dentition of individual IV-1 at age 3 yrs.

Figure 2.

Haplotype analysis by 6 polymorphic markers covering the 4q21-24 region. The following 6 polymorphic markers were able to distinguish between 5 and 10 different alleles in this kindred: D4S1592 (8 alleles), D4S392 (5), D4S2964 (6), D4S1534 (5), D4S414 (10), and D4S1572 (5). The alleles for each of these markers, determined for 24 members of the kindred, are shown below the symbol for each participant in the pedigree (**A**). The alleles for I-1 were deduced from his five offspring. Horizontal lines indicate recombination sites. A line passing through a number indicates that the side of the allele on which the crossover occurred could not be discerned. The diseased alleles are shaded. All of the 10 affected members have allele 4 for D4S1534, allele 6 for D4S414, and allele 2 for D4S1572. The corresponding linkage analysis calculations from MLINK are shown in **B**. The approximate distances of the 6 markers from the top of chromosome 4 are indicated in centiMorgans (cM). The columns on the right of the chromosome 4 map positions show the LOD scores, assuming recombination frequencies (θ) of zero, 0.1, 0.2, 0.3, and 0.4. Note that the LOD scores were above 3.0 (considered to be statistically significant) for the last 3 markers, when recombination frequencies of zero and 0.1 were assumed. The position of *DSPP* is between marker D4S2964 and D4S1534.

Table
Primers for *DMP1*, *MEPE*, and *DSPP* Characterizations

Primer Name	Primer Sequence	Annea *	Ext. T	Ext. t
(A) Primers used to amplify and sequence <i>DMP1</i>				
DMP1x1F	5'-TAGGGAAAATGTGCCTCTGC			
DMP1x1R	5'-TTCCATCTGCCTCCTGTTCT	55°C	72°C	30 s
DMP1x23F	5'-TCATGGACATTGGATGAATTT			
DMP1x23R	5'-GGAATCACAGCAGTATTTCTTC	55°C	72°C	40 s
DMP1x45F	5'-TTTCCTTTTGAACCATTTCA			
DMP1x45R	5'-GGACTCTTTAGCCAGCATAACTC	55°C	72°C	30 s
DMP1x6F1	5'-TTGGCAGCATTGAGTGAAGT			
DMP1x6R1	5'-TTTCCTACTGGGATGCTCCA	55°C	72°C	40 s
DMP1x6F2	5'-TGCAGAGTGATGACCCAGAG			
DMP1x6R2	5'-GCTGAGCTGCTGTGAGACTG	57°C	72°C	30 s
DMP1x6F3	5'-GGCCAACCTGTCATCTCAAG			
DMP1x6R3	5'-GTCCAGCAATTCCCTTCAGG	55°C	72°C	30 s
(B) Primers used to amplify and sequence <i>MEPE</i>				
MEPEx1F	5'-CCCCTTTTCTGGAACATTTGA			
MEPEx1R	5'-TCTAGAGCATGGTAACGCATTT	60°C	72°C	30 s
MEPEx2F	5'-TCCACACTCGGGTATAAGCA			
MEPEx2R	5'-GCTGCCTCTTGGTTTCTCA	60°C	72°C	30 s
MEPEx3F	5'-AAGCTGGCACTGTCTTTCTGT			
MEPEx3R	5'-CTCACTTAGCCCCATGAAGG	60°C	72°C	30 s
MEPEx4F1	5'-TCCATGAAACCTGATTTGACC			
MEPEx4R1	5'-AATACGATGGGTGCTTTTGC	60°C	72°C	30 s
MEPEx4F2	5'-AAACTCCTGGGGGAAGAAAA			
MEPEx4R2	5'-CTGCCTTTGCCATTTTATAGG	55°C	72°C	30 s
MEPEx4F3	5'-AGGGATGAAACTGCGAAAGA			
MEPEx4R3	5'-CCTCTCTTCACTGGTTCAGC	60°C	72°C	30 s
MEPEx4F4	5'-GAAAGGCTCCTGGGGTAGAC			
MEPEx4R4	5'-TGTGCTGGAGGCAGAACTAA	60°C	72°C	30 s
(C) Primers used to amplify and sequence <i>DSPP</i>				
DSPPx1F	5'-TCACCAAGTGAAGGAAGTGG			
DSPPx1R	5'-AAAGCCCAAGGTGGATTTTT	56°C	68°C	30 s
DSPPx2F	5'-GATGTCCCCATAACCACACC			
DSPPx2R	5'-CTCCATGACTTCTGGGCATT	56°C	68°C	30 s
DSPPx34F	5'-CAAGCCCTGTAAAGAAGCCACT			
DSPPx34R	5'-ACATGGATGCTTGTCATGGT	58°C	68°C	90 s
DSPPx5F	5'-CCTATGGCAACTTTTCCCAGT			
DSPPx5R	5'-TGTCATTGTCATCATTTCCCAT	56°C	68°C	30 s
DSPP34SF1	5'-GGACCATGGGAAAGAAGATG			
DSPP34SF2	5'-GCATCCAGGGACAAGTAAGC			
DSPP34SR1	5'-CATTCCCCTTCTCCCTTGTA			
DSPP34SR2	5'-CCTCGTTTCTACAGGAATTCTCA			
DSPP34SR3	5'-TGGAGGTCTTGTCTCCATCA			

* Annea = annealing temperature; Ext. T = extension temperature; Ext. t = extension time.