



Published in final edited form as:

J Endod. 2008 December ; 34(12): 1470–1473. doi:10.1016/j.joen.2008.08.027.

A Dentin Sialophosphoprotein Mutation That Partially Disrupts a Splice Acceptor Site Causes Type II Dentin Dysplasia

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Abstract

The dentin sialophosphoprotein (*DSPP*) gene on chromosome 4q21.3 encodes the major noncollagenous protein in tooth dentin. *DSPP* mutations are the principal cause of dentin dysplasia type II, dentinogenesis imperfecta type II, and dentinogenesis imperfecta type III. We have identified a *DSPP* splice junction mutation (IVS2-6T>G) in a family with dentin dysplasia type II. The primary dentition is discolored brown with severe attrition. The mildly discolored permanent dentition has thistle-shaped pulp chambers, pulp stones, and eventual pulp obliteration. The mutation is in the sixth nucleotide from the end of intron 2, perfectly segregates with the disease phenotype, and is absent in 200 normal control chromosomes. An *in vitro* splicing assay shows that pre-mRNA splicing of the mutant allele generates wild-type mRNA and mRNA lacking exon 3 in approximately equal amounts. Skipping exon 3 might interfere with signal peptide cleavage, causing endoplasmic reticulum stress, and also reduce *DSPP* secretion, leading to haploinsufficiency.

Keywords

Dentin; dentin dysplasia type II; dentinogenesis imperfecta; *DSPP*; pre-mRNA splicing

Pulpal obliteration can complicate the endodontic treatment of teeth, causing treatment failures and subsequent extraction of the involved teeth. Idiopathic or pathologic conditions can induce generalized or localized pulpal obliteration. Generalized pulpal pathosis is also a feature in certain genetic diseases that affect dentin and pulp tissue (1).

Hereditary dentin defects are separated into 2 main categories, dentinogenesis imperfecta (DGI) and dentin dysplasia (DD) (2). DGI type I is a syndromic phenotype and is synonymous with osteogenesis imperfecta (OI) (3). The nondental phenotype in OI is sometimes subtle (4), and dentists need to be vigilant to avoid making an incorrect diagnosis of DD type II or DGI type II (5). DGI type II is characterized by amber discoloration of the dentition, severe attrition of the teeth, bulbous crowns, and early pulpal obliteration. DGI type III, which is

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believed to be distinct from DGI type II, is now considered as a more severe form of DGI type II (6). DD type I is characterized by normal-looking crowns with short roots with crescent-shaped pulpal remnants (7). DD type II is similar to DGI type II in the deciduous dentition but minimally affects the permanent dentition. The crowns of the permanent teeth are normal-looking or discolored gray, with thistle-shaped pulp chambers and pulp stones that eventually obliterate the pulp chamber (8). DD type II is rare, compared with DGI type II.

Defects in the dentin sialophosphoprotein (*DSPP*) gene cause DGI type II and type III and DD type II (9-19). Only 4 mutations have been reported to cause DD type II, 1 missense mutation in the signal peptide and 3 frameshift mutations in the DPP coding region. Because of the rarity of DD type II and the lack of supporting data of known mutations, its molecular genetic etiology and proper genotype-phenotype correlations are still unknown. Here we report clinical findings and the results of mutational analysis of a Hispanic family with DD type II.

Materials and Methods

Identification of a Kindred and Enrollment of Human Subjects

This study was independently reviewed and approved by the Institutional Review Board at the University of Michigan and the Seoul National University Dental Hospital. The experiments were undertaken with the understanding and written consent of each subject according to the Declaration of Helsinki. The proband was a 4-year-old boy who presented with the dental features of DGI type II or DD type II. Five members of the family consented to participate in this study and contributed samples for genomic DNA analyses. All participating members received clinical and radiologic examinations.

Polymerase Chain Reaction and Sequencing

Genomic DNA was isolated from peripheral whole blood of participating family members (II-2, III-1, III-2, III-3, and III-4) by using the QIAamp DNA Blood Maxi Kit (Qiagen Inc, Valencia, CA). The purity and concentration of the DNA were quantitated by spectrophotometry, as measured by the OD₂₆₀/OD₂₈₀ ratio. Polymerase chain reaction (PCR) conditions and primer pairs used for PCR and sequencing were previously described (12). PCR amplification products were purified by QIAquick PCR Purification Kit and protocol (Qiagen Inc). DNA sequencing was performed at the DNA sequencing center (Macrogen, Seoul, Korea). All nucleotide numbering was determined by counting from the A of the ATG translation initiation codon of the human *DSPP* reference sequence (NM_014208.3).

In Vitro Splicing Assay

Genomic DNA of a normal control without any dental and general health problems was amplified with pfu enzyme (Elpisbio, Seoul, Korea) by using PCR primers (amplicon size, 2767 base pairs; sense, GGGGGCGGCCGCGGGCAAATGCTTACACATCA; antisense, AGGATCCATGATTTACATAAGAC) to include exons 2, 3, and 4 of *DSPP* gene. The amplification product was cloned into the pSPL3 splicing vector after double digesting with *NotI* and *BamHI* restriction endonucleases. PCR mutagenesis was performed to change T to G at the splicing acceptor site -6 position of intron 2. Sequences of normal and mutant pSPL3 vectors were confirmed by direct plasmid sequencing. Normal and mutant pSPL3 vectors were transfected into COS-7 cells, and total RNA was isolated after 36 hours. Reverse transcriptase-PCR (sense, TATTTTTGCATTTGGGCAGT; antisense, CCTACTTCTGCCCACTTAGAGC primers) was performed with HiPi PCR premix (Elpisbio). Amplification bands were excised from an agarose gel after electrophoresis, purified, and characterized by direct DNA sequencing as described above.

Results

Clinical Findings

Physical examination of the family members revealed no obvious non-oral abnormalities such as bone fragility or progressive hearing loss (Fig. 1A). The proband (III-4) was a 4-year-old boy who presented with the dental features of DGI type II or DD type II. His primary teeth were discolored brown and showed severe attrition (Fig. 2C). The dentition of his affected brother (III-1) looked normal; however, the panoramic radiograph showed features characteristic of DD type II (Fig. 2B, E). The premolar and canine teeth had thistle-shaped pulp chambers with pulp stones. The coronal pulp chambers of the maxillary anterior teeth were obliterated, and the radicular pulp chambers of the molar teeth, especially those of the first molars, were obliterated. His mother (II-2) had a mildly discolored dentition, with minor attrition and fractures along the incisal edges of her anterior teeth (Fig. 2A). The panoramic radiograph showed almost complete pulpal obliteration except for small pulpal remnants in several posterior teeth (Fig. 2D).

Mutation Analyses

Mutational analysis revealed a T to G change at the intron 2 splice acceptor site -6 position (g.1185T>G, IVS2-6T>G) of the *DSPP* gene (Fig. 1B, C). The identified mutation perfectly segregated with the disease phenotype in this family and furthermore was absent in 200 normal control chromosomes. An *in vitro* splicing assay showed that this mutation in the splicing acceptor site of intron 2 alters pre-mRNA processing and results in the deletion of exon 3 in the mutant mRNA, which is present in approximately equal amounts as the correctly spliced mRNA transcript (Fig. 1D).

Discussion

Currently 19 mutations in the *DSPP* gene, including 4 mutations causing DD type II, have been reported (Table 1). Several interesting patterns have emerged, but the genotype-phenotype relationship is not yet known. Most of the mutations in the 5' end of *DSPP* are single nucleotide substitutions near intron/exon junctions (15). It is difficult to predict with certainty what the effects of these mutations are in terms of *DSPP* expression. Retention of an intron would likely lead to degradation of the mRNA transcript by nonsense mediated mRNA decay (NMD), a mechanism that degrades mRNA harboring a premature translation termination codon (20). Degradation of the mRNA from the mutant *DSPP* allele would cause haploinsufficiency or a simple reduction in the quantity of normal mRNA and protein expressed. Mutations in the 3' end of *DSPP* are deletions and/or insertions that shift the reading frame in a way that replaces the C-terminus of DSPP with a long chain of extraneous amino acids (18,19). Interestingly, no mutations shift the reading frame in a way that simply truncates the protein (which would occur with shifts to the -2 reading frame), suggesting that such mutations do not cause a dominant dental phenotype.

In our kindred with DD type II, sequence analysis of the proband's DNA revealed a mutation (g.1185T>G, IVS2-6T>G) in the sixth nucleotide from the end of intron 2. This nucleotide is part of the 3' polypyrimidine tract that is necessary for proper pre-mRNA splicing (21). A polypyrimidine (C or T) tract longer than 6 nucleotides is necessary for splicing in mammalian species such as human, mouse, and dog (22). Computer splicing algorithms (www.fruitfly.org/seq_tools/splice.html) decreased the splice acceptor site prediction value of the mutant compared with the wild-type polypyrimidine tract from 0.97 to 0.74. The results of an *in vitro* splicing assay supported this prediction that the IVS2-6T>G alteration would diminish recognition of the end of intron 2 as a splice junction. RNA analysis showed normally spliced mRNA as well as mRNA with exon 3 deleted (Fig. 1D). This finding suggests that

disruption of the splice acceptor site by the IVS2-6T>G mutation is not complete but partial and does not result in intron retention. Skipping of exon 3 during pre-mRNA splicing deletes 28 amino acids (from Val¹⁸ through Gln⁴⁵; p.V18_Q45) from the N-terminal region of DSPP. Such an alteration could secondarily interfere with either the secretion of DSPP or cleavage of its signal peptide after Ala¹⁵. A percentage of the pre-mRNA from the diseased allele, however, would splice normally and combine with expression from the wild-type *DSPP* allele to produce more than half of the normal amount of DSPP protein in affected individuals. Previously, a point mutation in the *DSPP* signal peptide coding region (c.16T>G, p.Y6D) that was manifested as a DD type II phenotype was shown to reduce the secretion of DSPP as a result of partial failure of endoplasmic reticulum (ER) translocation (11). Perhaps the reduction of the normal amount of DSPP protein (about 25% reduction according to this study) results in the relatively mild phenotype of DD type II, whereas a larger reduction, as would be expected in haploinsufficiency, results in the more severe forms of inherited dentin defects (DGI type II and type III).

The data, however, are also consistent with a competing theory. Deletions or point mutations in or near the signal peptide coding region might affect normal signal peptide cleavage and interfere with protein folding and secretion, inducing an ER stress response and general cell pathology (23). It has even been suggested that all *DSPP* mutations yet reported cause their DD-II, DGI-II, and DGI-III phenotypes through dominant negative effects and not haploinsufficiency (18). The findings of our study indicate that exon skipping rather than intron retention is the more likely outcome of *DSPP* splice junction mutations, which is more consistent with the dominant negative explanation for the dental pathology. The effects of exon 3 deletion on the efficiency of signal peptide cleavage causing possible ER stress need to be studied further.

A better understanding of the pathogenesis of inherited diseases affecting dentin might improve the ability of dental practitioners to choose among treatment options in patients with *DSPP* mutations. Pathologic pulp chamber obliteration related to genetic conditions like those seen in our family with DD type II can be predicted at early stages before the development of pulp stones or pulp canal obliteration. Furthermore, mutational analysis would reveal genotype-phenotype correlations and lead us to a better understanding of dentin mineralization and homeostasis. The functional role of DSPP and its relationship to various transcription factors are critical to the success of regenerative treatment or gene therapy for dentin-pulp complex (24,25).

In conclusion, we have identified a novel mutation in the splice acceptor site of intron 2 of the *DSPP* gene in a family with DD type II. The partial disruption of splicing efficiency resulted in normal mRNA transcripts along with an equal quantity of exon 3 deleted transcripts. The functional role of the DSPP protein as well as the effect of the *DSPP* mutations on protein expression needs to be further studied to better understand the role of DSPP in normal and pathologic dentin formation.

Acknowledgments

We would like to thank all the family members for their cooperation. We thank Dr Alessandro Stella, University of Bari, Italy, for the pSPL3 splicing vector used in this study. This research was supported by grant 03-2007-0080 from the Seoul National University Dental Hospital Research Funds (J.-W. Kim) and NIDCR/NIH grant DE015846 (J. P. Simmer).

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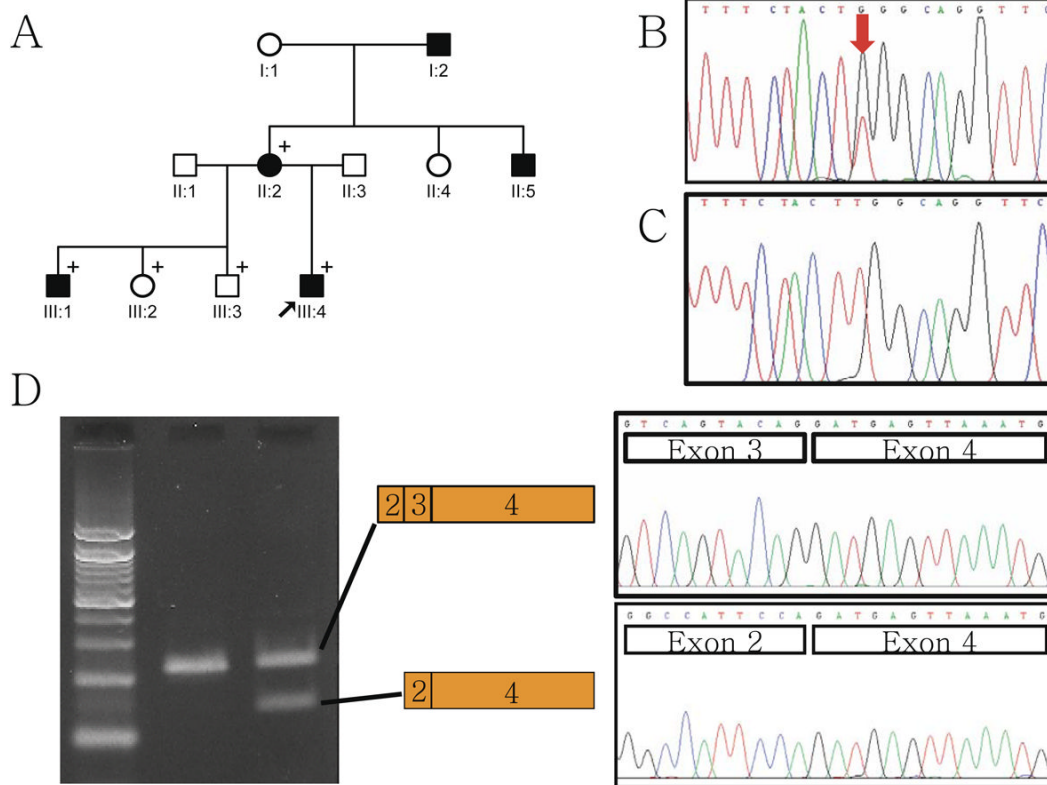


Figure 1. Pedigree and mutational analyses of the kindred. (A) Pedigree of the kindred. Black arrow indicates proband. A + identifies members recruited in this study. (B) DNA sequencing chromatogram of the affected individual III-4. Red arrow indicates the position of the mutation. (C) DNA sequencing chromatogram of the unaffected individual III-3. (D) Agarose gel image of *in vitro* splicing assay. Left lane is DNA size marker. Middle lane is a result of pSPL3 vector with normal insert. Right lane is a result of pSPL3 vector with mutated insert. Upper band in the right lane is normally spliced mRNA transcript, and lower band is abnormally spliced mRNA with exon 3 deletion.

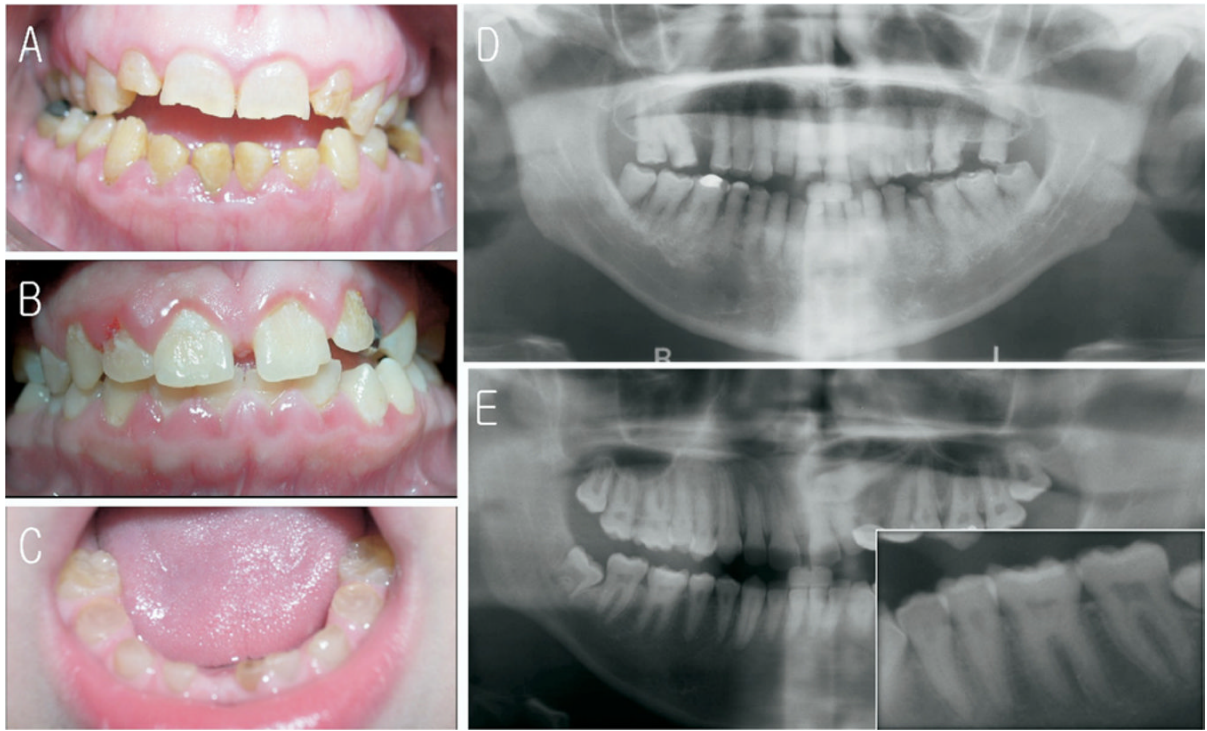


Figure 2.

Clinical photographs and radiographs. (A) Frontal view of affected individual II-2 at age 39. Note mild discoloration with minor attritions. (B) Frontal view of affected individual III-1 at age 15. Clinically permanent teeth look normal. (C) Frontal view of affected individual III-4 at age 4. Note brown discoloration and attrition. (D) Panoramic radiograph of affected individual II-2 at age 39. Note almost complete pulpal obliterations. (E) Panoramic radiograph of affected individual III-1 at age 15. The premolar and canine teeth have thistle-shaped pulp chambers with pulp stones. The inset is a magnified view of left mandibular premolars and molars. Note thistle-shaped pulp chambers and pulp stones.

TABLE 1
Summary of Mutations in *DSPP* Resulting in Hereditary Dentin Defects

	cDNA*	Protein†	Diagnosis	Reference
Exon 2	c.16T>G	p.Y6D	DD-II	Rajpar et al. (11)
	c.44C>T	p.A15V	DGI-II	Malmgren et al. (13)
	c.49C>A	p.P17T	DGI-II	Xiao et al. (9)
	c.49C>T	p.P17S	DGI-II	Hart and Hart (6), Zhang et al. (17)
Intron 2	c.52-6T>G	Normal + p.V18_Q45del	DD-II	This report
	c.52-3C>G	p.V18_Q45del	DGI-II	Kim et al. (12)
	c.52-3C>A	p.V18_Q45del	DGI-II	Holappa et al. (15)
Exon 3	c.52G>T	p.V18_Q45del or p.V18F	DGI-II, DGI-III	Xiao et al. (9), Kim et al. (14), Song et al. (16), Holappa et al. (15)
	c.133C>T	p.V18_Q45del or p.Q45X	DGI-II	Zhang et al. (10), Song et al. (16)
Intron 3	c.135 + 1G>A	p.V18_Q45del	DGI-II	Xiao et al. (9)
	c.135 + 1G>T	p.V18_Q45del	DGI-II	McKnight et al. (18)
Exon 5	c.1870_1873delTCAG	p.S624fsX1312	DD-II	McKnight et al. (18)
	c.1918_1921delTCAG	p.S640fsX1312	DD-II	McKnight et al. (18)
	c.2040delC	p.S680fsX1313	DD-II	Song et al. (19)
	c.2272delA	p.S758fsX1313	DGI-II	McKnight et al. (18)
	c.2525delG	p.S842fsX1313	DGI-II	McKnight et al. (18)
	c.2593delA	p.S865fsX1313	DGI-II	Song et al. (19)
	c.2684delG	p.S895fsX1313	DGI-II	Song et al. (19)
	c.3438delC	p.D1146fsX1313	DGI-II	Song et al. (19)
	c.3546_3550delTAGCAinsG	p.D1182fsX1312	DGI-II	Song et al. (19)

* Numbering assumes the A of the ATG start codon as nucleotide 1 on the basis of reference sequence NM_014208.3.

† These are mostly predictions.