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ORIGINAL ARTICLE

Identification of the DSPP mutation in a new kindred and phenotype–genotype correlation

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OBJECTIVE: Hereditary dentin defects can be grouped into three types of dentinogenesis imperfecta (DGI) and two types of dentin dysplasia. Tooth enamel is considered normal in patients with hereditary dentin defects, but is easily worn down and fractured due to *DSPP* mutation-induced altered dentin properties. The purposes of this study were to identify genetic cause of a family with type II DGI and enamel defects.

MATERIALS AND METHODS: We identified a family with type II DGI and a unique form of hypoplastic enamel defect affecting occlusal third of the crown. Family members were recruited for the genetic analysis and DNA was obtained from peripheral whole blood.

RESULTS: Mutational analysis revealed a T to A transversion in exon 3 of the *DSPP* (c.53T>A, p.V18D). Haplotype analysis showed that the same mutation arose separately in two different families having DGI with similar enamel defects, indicating that this phenotype is associated with this specific *DSPP* mutation. Clinical features suggest that enamel formation was affected in the affected individuals during early amelogenesis, in addition to the dentin defect.

CONCLUSIONS: We observed that a *DSPP* gene mutation not only influences dentinogenesis but also affects early stage amelogenesis.

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Keywords: dentin sialophosphoprotein; *DSPP*; dentinogenesis imperfecta; dentin dysplasia; enamel defect

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Introduction

Dentin is a major structural component of tooth and serves as the framework upon which enamel and cementum form. Dentin supports enamel, the hardest tissue in the human body, via mechanical stress distribution through well-organized dentinoenamel junctions. Without proper dentin support, enamel tends to wear easily and break down by fracture. Although dentin has similar mechanical properties and organic contents to those of bone, it has a unique microstructure with the long odontoblast processes remaining in the dentin matrix. This feature provides nutrient supplies and protects the tooth from harmful stimuli through nociceptive reception and tertiary dentin formation (Nanci, 2003).

Hereditary dentin defects include dentinogenesis imperfecta (DGI) and dentin dysplasia (DD) (Shields *et al*, 1973). DGI type I is a syndromic phenotype that can be seen in some forms of osteogenesis imperfecta. DGI type II is characterized by opalescent discoloration, bulbous crown shape, and pulpal obliteration. DGI type III was originally created to describe the unique phenotype (multiple pulp exposure and shell-like teeth) of the Brandywine tri-racial isolate in the US, but is now considered a severe form of DGI type II, not restricted to a specific cohort (Hart and Hart, 2007; Kim and Simmer, 2007). DD type II is characterized by the same phenotype as DGI type II in the deciduous dentition, but the permanent dentition shows normal to minimal tooth discoloration. However, the permanent dentition has a thistle tube-shaped pulp chamber and pulp stones. DD type I is rather unique in comparison with other types of hereditary dentin defects. Both dentitions exhibit normal color and shape, but radiologically, the roots are short and the pulp chambers are obliterated.

So far, the enamel itself has been thought to be normal in subjects with hereditary dentin defects. In this study, we identified a family with unique enamel defects in addition to DGI type II. Mutational analysis was

performed and the underlying mutation was identified. We also describe the clinical phenotype of other families having the same mutation.

Materials and methods

Enrollment of human subjects

The study protocol was reviewed and approved by the institutional review board at the Seoul National University Dental Hospital. The experiments were performed with the understanding and written consent of each participating subject according to the Declaration of Helsinki.

Primer design, polymerase chain reaction (PCR), and DNA sequencing

Genomic DNA was isolated from peripheral blood using the QuickGene DNA whole blood kit S with QuickGene-Mini80 equipment (Fujifilm, Tokyo, Japan). Conditions for the PCR and the oligonucleotide primer pairs used for *DSPP* PCR amplifications and DNA sequencing were previously described (Kim *et al*, 2004). PCR reactions were performed using HiPi DNA polymerase premix (ElpisBio, Daejeon, Korea). PCR products were purified according to the supplied protocol with the PCR Purification Kit (ElpisBio). DNA sequencing was performed at the DNA sequencing center (Macrogen, Seoul, Korea). All nucleotide numbering was counted from the A of the ATG translational initiation codon of the human *DSPP* reference sequence (NM_014208.3).

Genotype construction

Haplotype analysis was performed on the current family and the Korean family previously reported (Lee *et al*, 2009). For haplotype construction, PCR amplification was performed and the amplified product was cloned using Topcloner PCR cloning kit (Enzynomics, Seoul, Korea). The size of the amplified product was 1490 bp (sense: 5′-CAAGCCCTGTAAGAAGCCACT-3′, antisense: 5′-ACATGGATGCTTGTCATGGT-3′). Plasmid DNA of each clone was purified using a plasmid miniprep kit (ElpisBio) and sequenced. Sequencing results were analyzed using ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html) and the haplotype was drawn.

Results

Mutation results

Mutational analysis showed a T to A change (g.1198T > A, c.53T > A, p.V18D) in exon 3 of the *DSPP* gene (Figure 1a,c). This mutation was previously identified in two families with DGI type II (Kida *et al*, 2009; Lee *et al*, 2009). Haplotype analysis revealed that this mutation did not originate from a common ancestor (Figure 2).

Clinical findings

The proband (III:1) was 6 years old when she first presented to the Pediatric Dental Clinic at the Seoul National University Dental Hospital. Her dentition was amber-brown and the attrition of the deciduous teeth was severe. In addition to the typical characteristics of DGI type II, unique enamel defects were noted on the newly erupted anterior permanent teeth. The enamel was hypoplastic near the incisal edge of the anterior teeth. Defects could be identified in the radiographs even before eruption of teeth (Figure 3a). Similar enamel defects were found to be present in the canines and premolars during follow-up appointments (Figure 1d,e). The enamel defects were localized to the occlusal third of the crown. Intraoral radiographs revealed pulpal obliteration, reduced enamel density, and atypical enamel (Figure 3a).

Interestingly, these unique enamel defects were also noticed in another large family with the same mutation (Lee *et al*, 2009). All affected individuals in mixed dentition or young permanent dentition showed similar

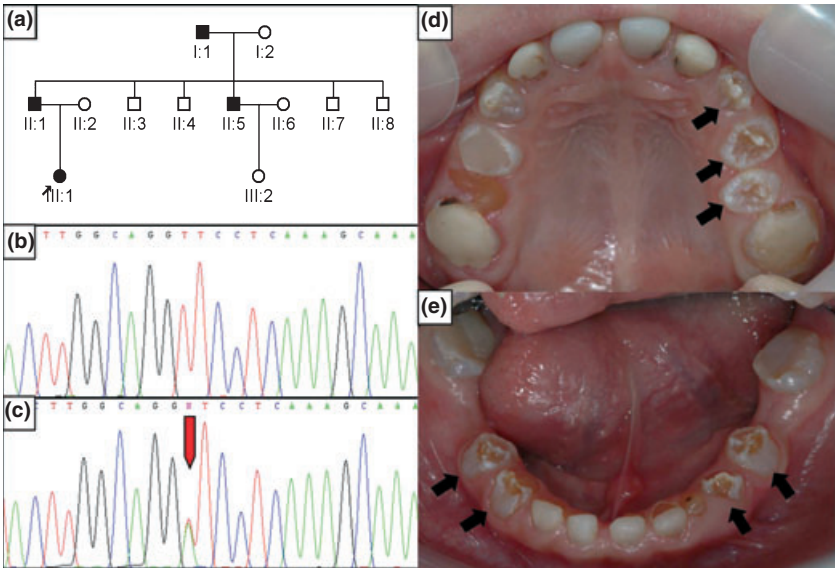

Figure 1 Pedigree and mutational analysis, and clinical photographs. (**a**) Pedigree of the proband's family. (**b, c**) DNA sequencing chromatogram of a normal control and an affected individual. The red arrow indicates the mutated nucleotide (g.1198T > A, c.53T > A). (**d, e**) Maxilla and mandibular clinical photographs of the proband taken at the age of 8.5 years. Enamel defects are indicated with black arrows

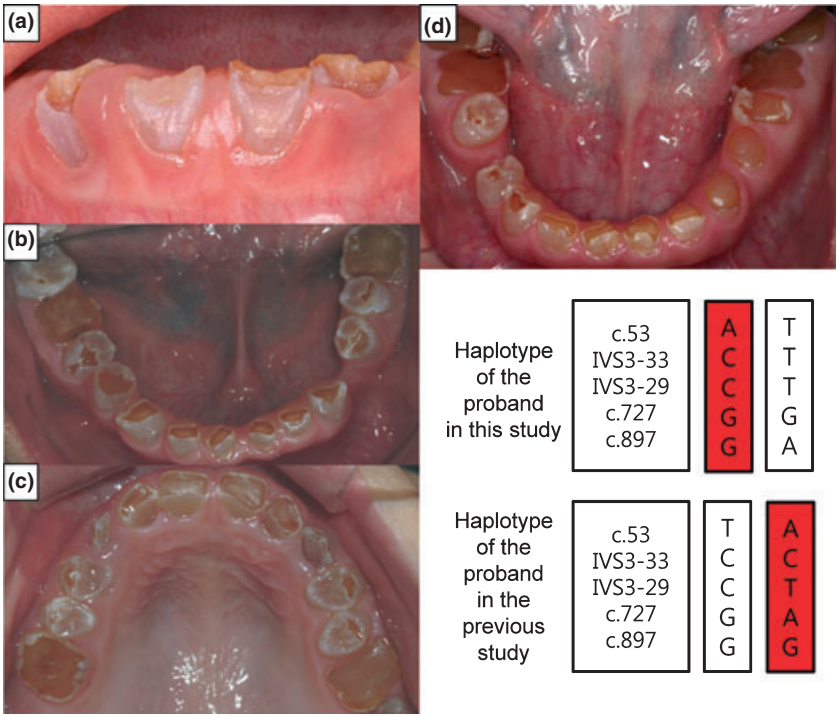

Figure 2 Clinical photographs and haplotype analysis. (**a**) Frontal photograph of the proband of the previous study (Lee *et al*, 2009). Enamel defects were noticed on the newly erupted anterior permanent teeth. (**b**) Mandibular clinical photographs of an affected individual (IV:7) from the previous family (Lee *et al*, 2009). (**c**) Maxillary clinical photographs of an affected individual (IV:8) from the previous family (Lee *et al*, 2009). (**d**) Mandibular clinical photographs of an affected individual (IV:17) from the previous family (Lee *et al*, 2009). Similar enamel defects were noticed on the permanent teeth. Haplotype analysis showed that the disease-causing allele is different and the mutation did not originate from a common ancestor. Alleles with red color represent ones harboring mutation (c.53T > A)

enamel defects (Figures 2 and 3b,c). Furthermore, this defect was also identified in a Japanese family with the same mutation caused spontaneously (Figure 4). Pregnancy and delivery was uneventful and there was no unhealthy condition, which could affect enamel formation in affected individuals in this and previous study.

Discussion

Genetic studies have identified the molecular genetic etiology of hereditary dentin defects. DGI type I is dental defect of osteogenesis imperfecta with variable penetrance and expressivity. Many mutations in *COL1A* or *COL1A2* have been identified in osteogenesis imperfecta patients with concurrent DGI (Pallos *et al*, 2001; De Coster *et al*, 2007; Barbirato *et al*, 2009).

The genetic etiology of DD type I is still unknown; however, many *DSPP* gene mutations have been identified in DGI type II, III, and DD type II (Xiao *et al*, 2001; Zhang *et al*, 2001; Rajpar *et al*, 2002; Kim *et al*, 2004, 2005; Malmgren *et al*, 2004; Holappa *et al*, 2006; Lee *et al*, 2008, 2009; McKnight *et al*, 2008a,b; Kida *et al*, 2009; Wang *et al*, 2009). Therefore, these diseases do not seem to be separate entities, but rather allelic ones with varying phenotypes according to the nature of the mutation. DGI type III is no longer considered as a phenotype specific to the Brandywine isolate because similar clinical features have been reported in families of different origins (Heimler *et al*, 1985; Sapir and Shapira, 2001). Furthermore, a genetic study revealed that the disease causing mutation in the Brandywine isolate is identical to *DSPP* gene mutation (c.49C > T, p.P17S) observed in a Chinese family having DGI type II (Hart and Hart, 2007; Zhang *et al*, 2007). DD type II is now considered as a mild form of hereditary dentin defect. With the finding of a signal peptide mutation (c.16T > G, p.Y6D) in a DD type II family, it has been theorized that a reduced amount of normal DSPP protein in the developing dentin matrix resulted in a mild clinical phenotype (Rajpar *et al*, 2002). Another study revealed a mutation (c.52-6T > G) partially affecting normal pre-mRNA splicing in a DD type II family (Lee *et al*, 2008). Success in sequencing DPP part having heavily repetitive sequence revealed three frameshift mutations in the N-terminal part of DPP in DD type II families (McKnight *et al*, 2008a,b; Song *et al*, 2008). Successful sequencing of DPP part presented a great advancement in the molecular genetic study of these diseases; however, further research is needed to fully understand the pathogenesis of *DSPP*-associated dentin defects and the genotype–phenotype correlation.

Interestingly, the proband of this study had a unique hypoplastic enamel defect. Furthermore, members of another family having the same mutation also had such an enamel defect (Lee *et al*, 2009). Because of the severe attrition, these enamel defects could not be identified in the older patients. However, every young affected individual in the previous study (Lee *et al*, 2009) had the same defect. Furthermore, the same enamel defect was identified in a Japanese family with the same mutation caused spontaneously (Kida *et al*, 2009). Because the localized hypoplastic enamel defect is confined to the occlusal third of the crown, it will be lost because of attrition during mastication. Haplotype analysis showed that the disease-causing mutation did not originate from a common ancestor. Based on clinical and radiologic examination, these defects did not appear to be the result of enamel attrition or fracture. The

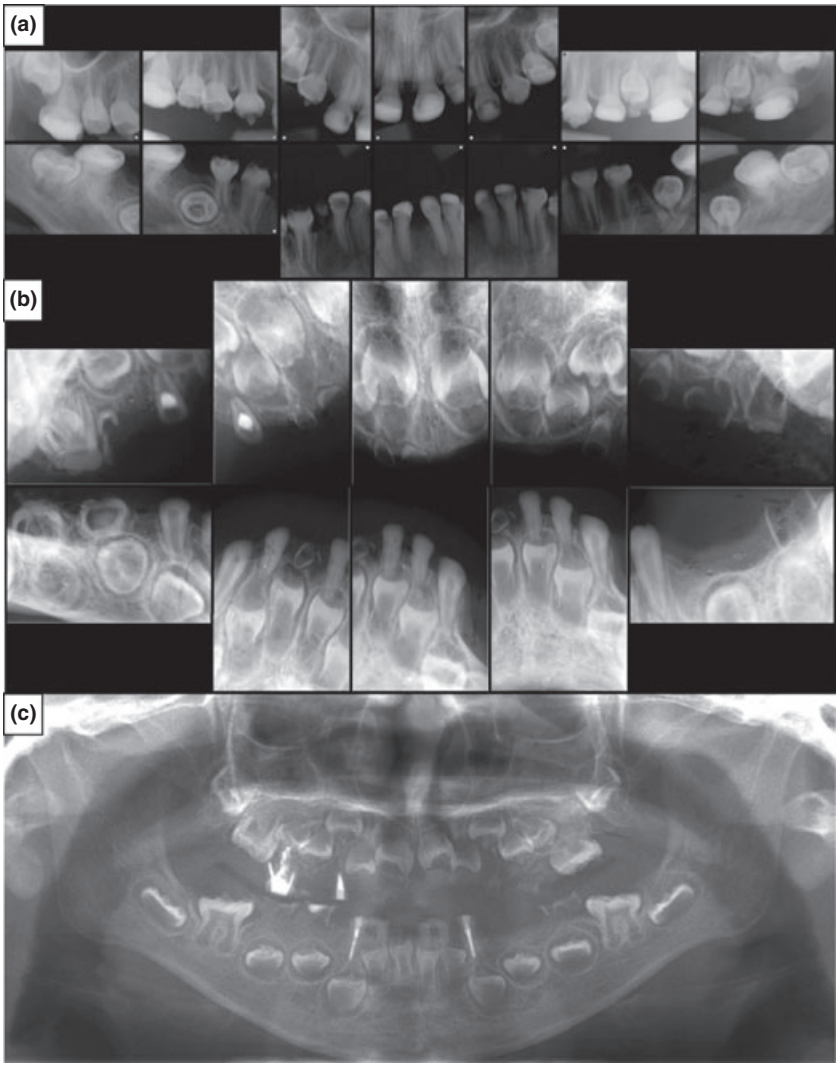

Figure 3 (**a**) Intraoral dental radiographs of the proband taken at the age 8.5 years. Mandibular anterior teeth and first permanent molars showed almost complete pulpal obliteration. Canines and premolars showed atypical enamel shape and reduced enamel radiopacity. (**b**) Intraoral dental radiographs of the proband in the previous study (Lee *et al*, 2009) taken at the age 4.5 years. Enamel defects are seen in developing permanent teeth. (**c**) Panoramic radiograph of the proband in the previous study (Kida *et al*, 2009). Hypoplastic enamel defects can be seen in developing permanent teeth

clinical features suggest that enamel formation was affected during early amelogenesis concurrently with the defects in dentinogenesis.

The mutation (g.1198T > A, c.53T > A, p.V18D) in this study has been demonstrated not to affect pre-mRNA splicing (Lee *et al*, 2009). The effect of this mutation is a missense mutation, replacing well-conserved hydrophobic Val^{18} with acidic Asp^{18}. DSPP has an *N*-terminal signal peptide consisting 15 amino acids. The location of the mutation is in the vicinity of the signal peptide cleavage site, so it may affect proper signal peptide cleavage (McKnight *et al*, 2008b). Dominant negative effect caused by the p.V18D might be responsible because this change could result in defective signal peptide cleavage and/or damaging cell's capability for proper protein secretion. No enamel defect was reported in the mutation changing Val^{18} with the other amino acid (p.V18F, c.52G > T) (Xiao *et al*, 2001; Kim *et al*, 2005). The severity of the mutation might be related with different phenotype; Valine is a non-polar neutral amino acid having hydropathy index of 4.2 and phenylalanine is also non-polar neutral amino acid with reduced hydropathy index of 2.8. However, aspartic acid is a polar negative amino acid with hydropathy index of −3.5. So the consequence of the p.V18D would be more severe than that of p.V18F.

Enamel and dentin are formed as a result of a series of ectodermal and ectomesenchymal interactions, the last of which consists of enamel formation on the dentin matrix (Thesleff, 2003). *DSPP* is transiently expressed in the presecretory ameloblast and continuously expressed in the odontoblasts (Begue-Kirn *et al*, 1998). *Dspp*-knockout mouse study showed that Dspp is not required for the nucleation of enamel crystals (Sreenath *et al*, 2003). However, it is possible that ameloblast functions at the early stage of amelogenesis are influenced by abnormal *DSPP* gene products. The reason that the defects were localized to the occlusal third is not known yet. But we think certain disturbance in the ameloblast is caused by this mutation during and/or just after DSPP expression in the preameloblast. So the enamel defect in this study could be attributed to the residual effect of ameloblast pathology initiated in preameloblast stage. The other possibility is that even if wild-type DSPP is not necessary for the enamel formation (as in the case of knockout mouse), a severe form of mutant DSPP (like

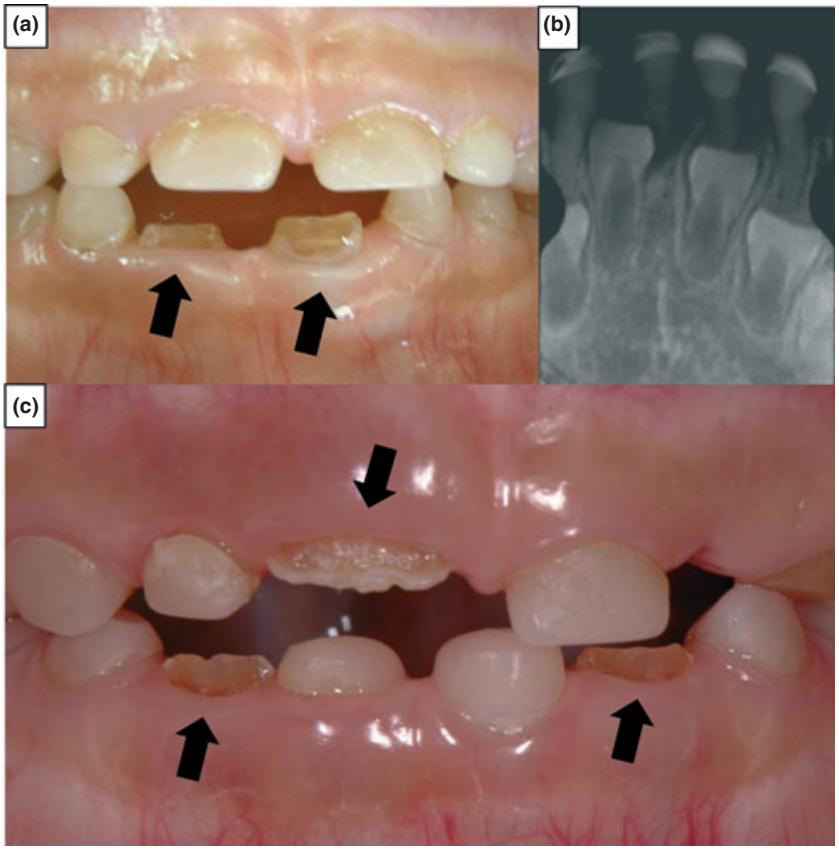

Figure 4 (**a**) Frontal photograph and intraoral radiograph of the proband in the previous study (Kida *et al*, 2009). Note the enamel defect (black arrows) in the erupting and developing mandibular incisors. (**b**) Frontal photograph and intraoral radiograph of the proband in the previous study (Kida *et al*, 2009). Enamel defects are seen in developing permanent teeth. (**c**) Frontal photograph of the proband in the previous study (Kida *et al*, 2009). Note the enamel defect (black arrows) in the erupting incisors

in this study) may interfere with the enamel formation. Taken together, it is possible that the specific mutation(s) in the *DSPP* gene could also deteriorate normal enamel formation by impairing the action of ameloblasts, in addition to interfering with dentin formation by the odontoblasts.

In summary, we have identified a mutation in exon 3 of the *DSPP* gene which resulted in DGI type II with a unique hypoplastic enamel defect. Functional characterization of DSPP and the effects of mutations should be explored further for a better understanding of dentinogenesis and to elucidate genotype–phenotype correlations.

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Author contributions

Sook-Kyung Lee, Kyung-Eun Lee, Yun-Hee Hwang, and Miyuki Kida performed experiments. Miyuki Kida, Tomonori Tsutsumi, and Jung-Wook Kim collected and analyzed patient information. Tadashi Ariga, Joo-Cheol Park, and Jung-Wook Kim designed the study and prepared the manuscript.

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