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ORIGINAL ARTICLE

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Dental properties, ultrastructure, and pulp cells associated with a novel *DSPP* mutation

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Objective: To investigate physical characteristics and behaviours of dental pulp cells of teeth isolated from a dentinogenesis imperfecta (DGI) patient with a novel dentin sialophosphoprotein (*DSPP*) mutation.

Subjects and Methods: Whole exome and Sanger sequencing were employed to identify mutations. Physical characteristics of the teeth were examined. Pulp cells' behaviours including cell proliferation, colony-forming unit, osteogenic differentiation, pluripotent markers, and mesenchymal stem cell markers were investigated.

Results: The proband had opalescent brown primary teeth with extensive loss of enamel. Mutation analysis revealed a novel heterozygous 4-bp deletion, c.1915_1918delAAGT (p.K639QfsX674), in exon 5 of the *DSPP* associated with DGI. Analysis of the extracted primary incisor demonstrated a decrease in brightness but an increase in yellow and red chroma. The dentin showed reduced mineral density. The dentinal tubules were present in the predentin, but progressively collapsed in the dentin. The pulp cells exhibited markedly reduced CD105 expression, decreased cell proliferation, and smaller colony-forming units.

Conclusions: We identified a novel mutation in the *DSPP* gene which disturbed dentin characteristics and pulp cells' behaviours. Our study expands the mutation spectrum and understanding of pathologic dentin phenotypes related to the frameshift deletion in the dentin phosphoprotein (DPP) region of the *DSPP* gene.

KEYWORDS
dental pulp cells, dentin sialophosphoprotein, dentinal tubules, dentinogenesis imperfecta, exome sequencing

1 | INTRODUCTION

Dentinogenesis imperfecta (DGI) is the most common genetic disorder of the dentin with reported incidence between 1 in 6,000 and 8,000 (Kim & Simmer, 2007). The affected teeth exhibit a yellow-brown opalescent colour, bulbous crown with constricted short roots, and obliterated pulp chamber (Levin, Leaf, Jelmini, Rose, & Rosenbaum, 1983). Dentinogenesis imperfecta can be either a part of a syndrome or isolated. Syndromic DGI is usually a feature of osteogenesis imperfecta, commonly caused by mutations in the genes encoding collagen type 1, *COL1A1* and *COL1A2*. Non-syndromic or isolated DGI is caused by heterozygous mutations in the dentin sialophosphoprotein (*DSPP*) gene (Kim & Simmer, 2007; O'Connell & Marini, 1999). *Dspp* null mice displayed dentin malformations similar to phenotypes of human DGI suggesting the importance of *DSPP* in dentinogenesis (Sreenath et al., 2003).

Dentin is a complex structure consisting of inorganic hydroxyapatite crystals and organic extracellular matrix. The type I collagen

and the non-collagenous proteins (NCPs) predominantly the DSPP in the extracellular matrix form a main framework for growth and mineralisation of the dentin (Butler & Ritchie, 1995). The *DSPP* mRNA is translated into a single protein, DSPP which is cleaved into two major NCPs, dentin sialoprotein (DSP) and dentin phosphoprotein (DPP) (MacDougall et al., 1997; Sreenath, Cho, MacDougall, & Kulkarni, 1999). Expression of DSP in the *Dspp* null mice partially rescued the null phenotype in terms of predentin width, dentin volume, and incidence of pulp exposure (Suzuki et al., 2009). The sequential extraction of NCPs showed the presence of DSP in the extract from predentin while DPP existed in the extract of the inorganic phase of dentin (Huang et al., 2008). Both proteins are sequentially expressed and play specific roles during dentinogenesis.

Human dental pulp stem cells (hDPSCs) constitute unique mesenchymal stem cells with self-renewal capability, multilineage differentiation, and clonogenic efficiency (Gronthos et al., 2002). DPSCs have an ability to regenerate a dentin-like mineralised tissue lined with odontoblast-like cells and pulp-like fibrous tissues which are important for dentin repair (Gronthos, Mankani, Brahim, Robey, & Shi, 2000). *Dspp* null mice displayed defective dentin and changes of *Runx2*, *Gli1*, *Numb*, and *Notch* expression in the dental pulp cells and odontoblasts suggesting the role of *DSPP* in the development of dentin-pulp complex (Chen, Zhang, Ramachandran, & George, 2016). Patients with DGI have suffered from severe tooth deterioration, multiple pulp exposure, pulp necrosis, and periapical infections. Although dentin defects have often been linked with pulp pathology in DGI, no previous studies have investigated the role of dental pulp cells associated with particular *DSPP* mutations contributing to DGI phenotype. This study performed comprehensive analyses of oro-dental phenotypes, genetic mutation, physical characteristics of DGI tooth, and dental pulp cells' behaviours associated with non-syndromic DGI.

2 | MATERIALS AND METHODS

2.1 | Enrolment of human subjects

A Thai boy with isolated DGI was recruited in this study. The study protocol was reviewed and approved by the Human Ethical Review Board at the Faculty of Dentistry, Chulalongkorn University, Thailand, and adhered to the Tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants. Clinical and radiographic examinations were performed. Blood and tooth samples were collected.

2.2 | Genetic analysis

Blood samples were collected from the proband, his mother, and his stepfather. The proband's genomic DNA was extracted from peripheral blood leucocytes and sent to Macrogen, Inc. (Seoul, Korea). Exome and Sanger sequencing were performed as described previously (Porntaveetus, Srichomthong, Ohazama, Suphapeetiporn, & Shotelersuk, 2017). The DNA sample was prepared as an Illumina sequencing library. The sequencing libraries were enriched by SureSelect Human All Exon V5 (Agilent Technologies, Santa Clara, CA) and were sequenced onto Hiseq 4,000 (Illumina, San Diago, CA). The raw data per exome were mapped to the human reference genome hg19 using Burrows–Wheeler Aligner (BWA). Variant calling was performed using GATK with HaplotypeCaller. Finally, SNVs and Indels were annotated using SnpEff and annotation databases, dbpSNP 142, 1,000 Genomes Project, ClinVar, and ESP. The variants were subsequently filtered out if they were present in our in-house database of 500 unrelated Thai exomes. The variants would be called novel if they were not listed in the Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac/index.php) and the Exome Aggregation Consortium database (exac.broadinstitute.org).

2.3 | Physical characterisation

Dentin colour was measured as Hunter l, a, b colour scale by ShadeEye-NCC® (Shofu Inc., Japan). Mineral density was determined using microcomputerised tomographic machine (μCT35, Scanco Medical, Switzerland). Dentin ultrastructure was examined using scanning electron microscope (SEM; Quanta 250, FEI, Hillsboro, OR). Subsequently, specimens were decalcified, sectioned, and stained with haematoxylin and eosin and Masson's trichrome.

2.4 | Cell culture

Cells were isolated from the remaining dental pulp tissues of an extracted deciduous incisor from the proband and unaffected controls. Tissue explant technique was employed for cell isolation according to previous publications (Nowwarote, Pavasant, & Osathanon, 2015; Nowwarote, Sukarawan, Pavasant, & Osathanon, 2017). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco®) containing 10% foetal bovine serum (FBS, Gibco®, USA), 100 unit/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml amphotericin B, 2 mM l-glutamine (Glutamax®) at 37°C in 5%CO_2. Cells at passages 3–5 were used in the experiments.

2.5 | Flow cytometry

Cells were stained with FITC-conjugated anti-CD44 antibody (BD Biosciences Pharmingen), PerCP-CyTM5.5-conjugated anti-CD90 antibody (BD Biosciences Pharmingen), and PE-conjugated anti-CD105 antibody (BD Biosciences Pharmingen). The expression was analysed using FACSCalibur (BD Bioscience).

2.6 | Cell proliferation assay

Cells were seeded at a density of 12,500 cells per well in a 24-well plate and treated with 1 mg/ml MTT solution (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, USB Corporation, Cleveland, OH) for 15 min at 37°C. Formazan crystals were solubilised using DMSO and glycine buffer. The absorbance was examined at 540 mm with a microplate reader.

2.7 | Colony-forming assay

Cells were seeded in 60 mm-diameter tissue culture dishes at a density of 500 cells/dish. Cells were maintained in growth medium for 14 days and subsequently fixed with 10% formalin and stained with Coomassie blue.

2.8 | Osteogenic differentiation

Cells were maintained in growth medium supplemented with 50 μg/ml ascorbic acid, 10 mM beta-glycerophosphate, and 100 nM dexamethasone. Mineral deposition was examined by alizarin red S staining according to previous publications (Nowwarote et al., 2015, 2017). The expression of osteogenic marker genes was evaluated using quantitative polymerase chain reaction.

2.9 | Polymerase Chain Reaction (PCR)

Total RNA was extracted with RiboEx total RNA isolation solution (GeneAll, Seoul, South Korea) and quantified using a Nanodrop®. RNA samples (1 μg) were converted to complementary DNA using the ImPromII kit (Promega, Madison, WI). Semi-quantitative PCR was performed in a thermocycling machine using Taq polymerase (Invitrogen). The product was electrophoresed in 1.8% agarose gel and visualised by ethidium bromide staining. For quantitative real-time PCR, the target genes were amplified using FastStart Essential DNA Green Master (Roche Diagnostic, USA) on MiniOpticon real-time PCR system (Bio-Rad). The expression value was normalised to *GAPDH* expression and the mRNA expression of cells cultured in growth medium. Primer sequences are shown in Table S1.

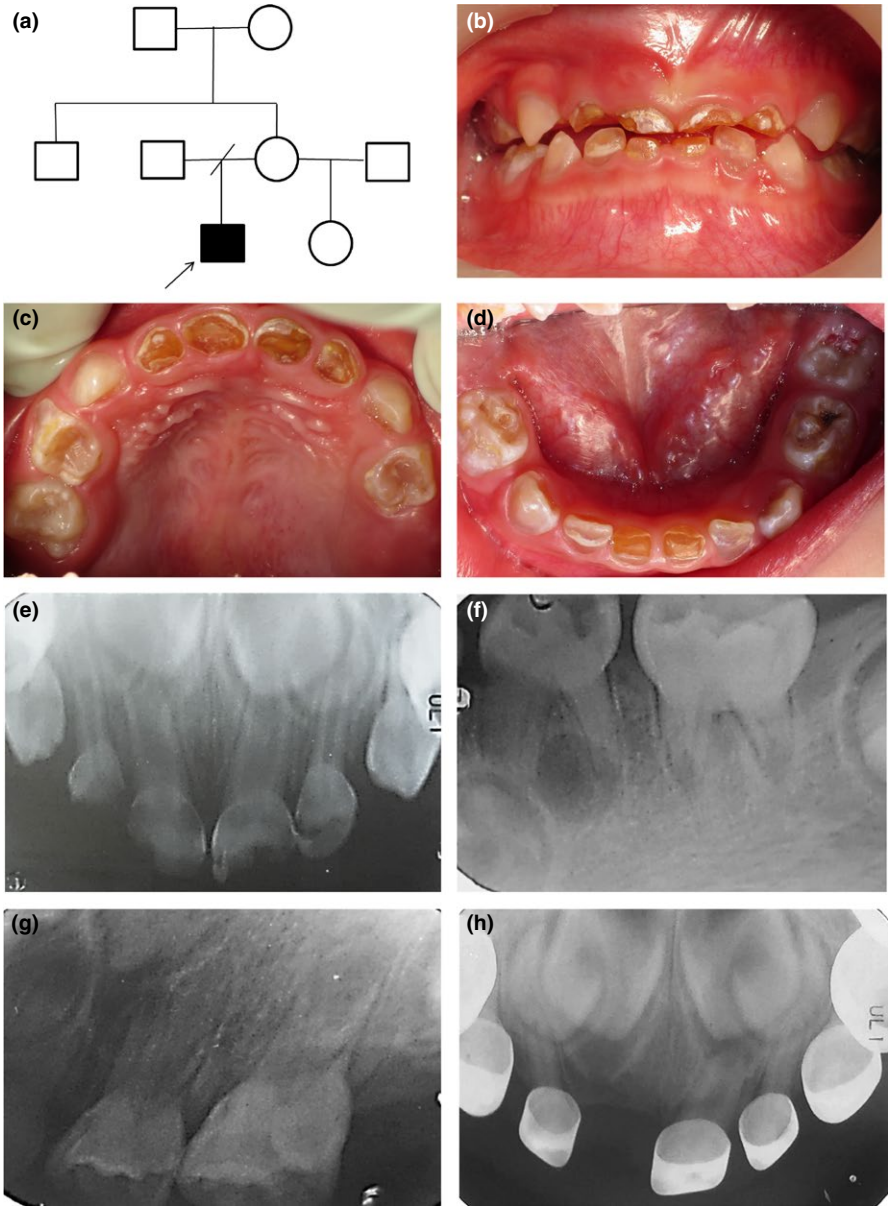

FIGURE 1 Pedigree, clinical, and radiographic manifestations of the proband. (a) Diagram demonstrates a pedigree of the proband's family. The blackened symbol represents the clinically affected individual. (b-d) Frontal, maxillary, and mandibular clinical photographs of the proband showed severe discolouration and deteriorated primary teeth at 2 years of age. (e-g) Dental periapical radiographs demonstrated the maxillary anterior teeth with large pulp cavities (e), and the mandibular and maxillary posterior teeth with narrow pulp (g,h). (h) Radiographic images at one-year recall illustrated the teeth restored with stainless steel crowns. The primary maxillary right central incisor was extracted [Colour figure can be viewed at wileyonlinelibrary.com]

3 | RESULTS

3.1 | Clinical and radiographic investigations

The proband presented at two and a half years of age. His parents were non-consanguineous. His mother was healthy. His biological father was not available for examination (Figure 1a). The proband's primary dentition exhibited generalised dark yellow-brown discolouration, extensive enamel deterioration, and dental caries with multiple exposures of remaining dentin (Figure 1b-d). The enamel and dentin were rapidly deteriorated soon after tooth eruption. Radiographic examinations revealed severe destruction of all four maxillary incisors. The dental pulps of maxillary incisors were large while those of molars were almost obliterated (Figure 1e-g). The dental treatment was performed under general anaesthesia. The maxillary right central incisor was extracted due to sinus opening at labial mucosa. The remaining teeth were restored with stainless steel crowns cemented with glass ionomer cement (CX-Plus; Shofu, Kyoto, Japan). The second molars were sealed with glass ionomer cement (GC Fuji VII) at a 6 month recall visit. All teeth were in good condition at 1-year recall (Figure 1h).

3.2 | Analysis of physical characteristics of the DGI tooth

The extracted incisor was further evaluated for ultrastructure and physical properties compared with teeth from unaffected controls. The DGI tooth showed opalescent dentin and extensive deterioration of the enamel and dentin (Figure 2a,b). Micro-CT revealed an uneven enamel surface, a large cavity involving the tooth crown, and a curved root (Figure 2c). Cross section showed a large dental pulp cavity containing a calcified mass (Figure 2d). Under scanning electron microscope, the proband's tooth demonstrated amorphous dentin without apparent dentinal tubules (Figure 2e,f), compared to the organised tubules found in a control tooth (Figure 2g,h). Histological sections of DGI tooth demonstrated that the dentinal tubules were misshaped, short, irregular, and reduced in number and the collagenous network was disorganised (Figure 2i,j), compared to those of the control (Figure 2k,l). Notably, the dentinal tubules of DGI tooth were found in the predentin, but became sparsely scattered and not apparent in the rest of the dentin, suggesting the collapse or obliteration of the tubules during dentinogenesis.

The dentin colour of DGI tooth appeared to be darker than the control dentin as the Hunter colour scale L was 57.8 and 89.4 for DGI dentin and the control dentin, respectively. In addition, DGI dentin appeared redder and yellower than the control (the values of dentin in DGI and control tooth were 0.7 and -1.2; b values were 16.0 and 10.2, respectively). Microcomputerised tomography analysis showed reduced mineral density in the DGI dentin (865.407 mg HA/cm^2) compared to the control (1,055.137 mg HA/cm^2). The mineral density of the enamel of DGI teeth (2083.856 mg HA/cm^2) was comparable to the control (2050.560 mg HA/cm^2). The pulp stone observed in DGI tooth showed the mineral density of 831.93 mg HA/cm^2.

3.3 | Genetic investigation

Exome sequencing revealed a novel heterozygous frameshift deletion, c.1915_1918delAAGT (p.K639QfsX674), in exon 5 of the *DSPP* gene (NM_014208.3) (Figure 3a,b). The variant was not identified in the mother. As the proband's biological father is not available, the heterozygous frameshift mutation could suggest an autosomal dominant inheritance or a *de novo* mutation. The mutation was located in the DPP domain which contained many repetitive DS and DSS motifs (Figure 3c).

3.4 | Characterisation of cells isolated from DGI pulp tissues

Cells isolated from the dental pulp tissue of the extracted maxillary right central incisor (referred as DGI cells) were characterised compared with those isolated from unaffected controls. DGI cells expressed mRNA of pluripotent markers (*OCT4* and *NANOG*) and mesenchymal stem cell markers (*CD44*, and *CD73*) similar to those cells from healthy donors (Figure 4a). For surface marker expression, DGI cells exhibited similar levels of CD44 and CD90 compared with the controls; however, a marked reduction in CD105 expression was observed in DGI cells (Figure 4b). Furthermore, DGI cells had a slight decrease in cell number at day 7 compared with the controls (Figure 4c). Although colony-forming unit ability was comparable between the DGI cells and the controls, colonies of DGI cells were relatively smaller in size compared with those of the controls (Figure 4d).

Osteogenic differentiation potential was investigated by culturing cells in osteogenic induction (OM) medium. After maintaining cells for 14 days, mineral deposition was evaluated using alizarin red S staining (Figure 4e,f). The mRNA expression of early osteogenic marker genes, *ALP* and *RUNX2*, was evaluated using real-time quantitative PCR at day 7 after induction. The results demonstrated that cells isolated from the dental pulp of the DGI-affected tooth were able to mineralise comparable to the control cells (Figure 4e). The upregulation of *ALP* and *RUNX2* mRNA expression was observed in osteogenic medium in cells isolated from the DGI-affected tooth and the control teeth (Figure 4f). DGI cells showed reduced expression of *RUNX2* in OM medium compared to the control donor cells (Figure 4f).

4 | DISCUSSION

Our proband presented with severe phenotypes of DGI in primary dentition including defective dentine with dark brown opalescent colour, broken enamel, and rapid progressive attrition of the teeth. The lower L* value and higher a* and b* values detected in the DGI tooth compared to those of controls suggest a decreased luminosity but enhanced chromaticity (yellowness and redness) which could contribute to the opalescent colour of DGI dentine. Radiologically, DGI teeth showed bulbous crowns with cervical constrictions, small and narrow roots, and large pulp chambers and root canals of the anterior teeth but almost obliterated those of the posterior teeth. A calcified mass

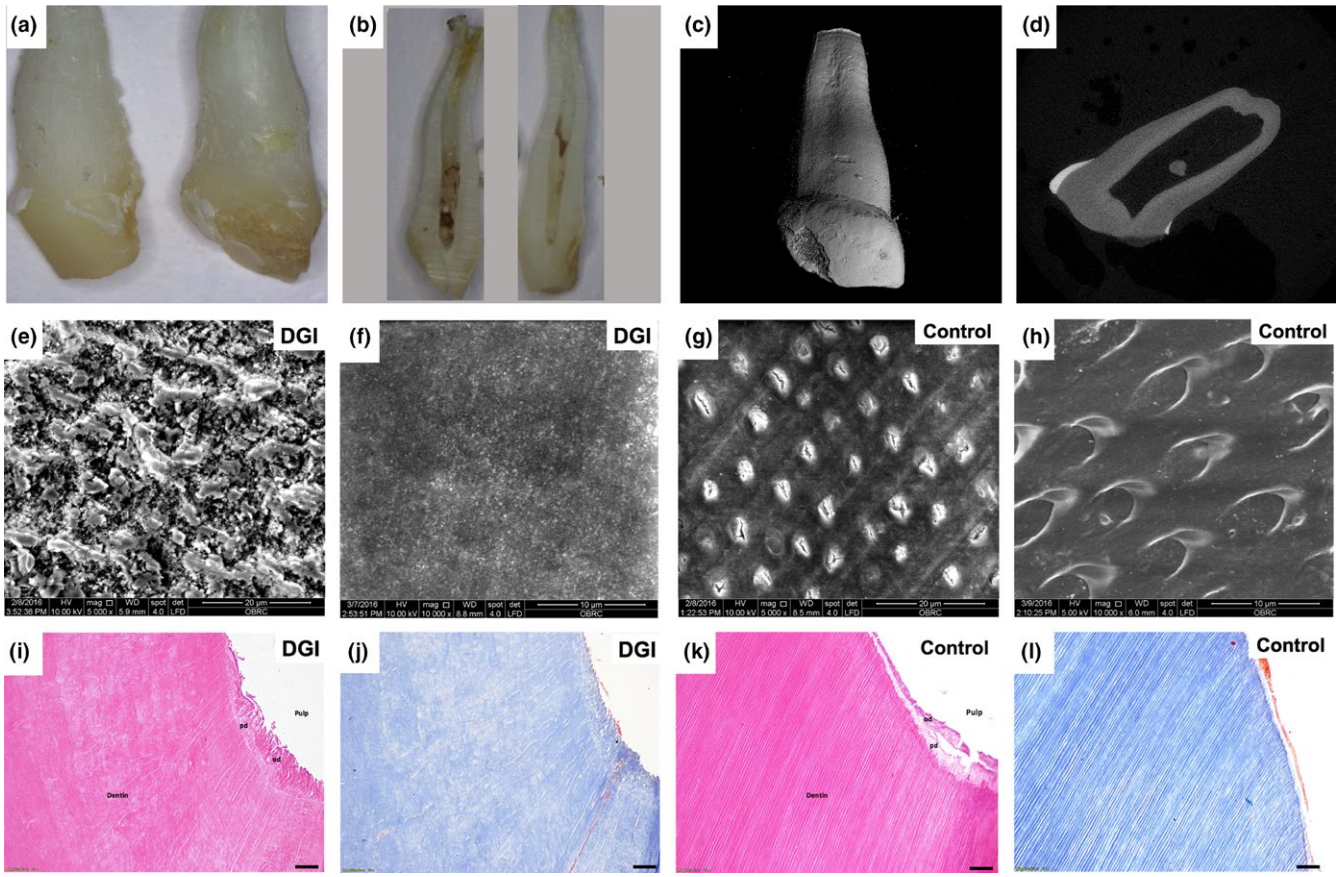

FIGURE 2 Ultrastructural analysis of the proband's primary maxillary incisor. (a, b) Opalescent dentin and extensive deterioration of the enamel and dentin were observed. (c) Three-dimensional illustration of micro-CT demonstrated irregular enamel surface and a large cavity involving the enamel and dentin. (d) Micro-CT cross section of the tooth showed a calcified mass in the pulp cavity. (e,f) Scanning electron microscopic images revealed amorphous dentin without dentinal tubules in the proband. (g,h) The control dentin showed the typical structure of organised dentinal tubules. (i-l) Histological sections showed scattered, irregular, and reduced dentinal tubules and collagen fibrils in the proband (i,j), compared to the control (k,l). (e, g), unetched condition; (f,h), 37% phosphoric acid etched condition; (i,k), haematoxylin and eosin; (j,l), Masson's trichrome; pd, predentin; od, odontoblasts. Scale bars = 50 μm [Colour figure can be viewed at wileyonlinelibrary.com]

was observed in the pulp cavity. The mineral density was reduced. Histologically, the dentine of an affected tooth showed a complete lack of dentinal tubules with less collagen deposition. These illustrate several anomalies associated with DGI.

Mutations in the *DSPP* gene are responsible for non-syndromic hereditary DGI (Malmgren, Lindskog, Elgadi, & Norgren, 2004; Xiao et al., 2001; Zhang et al., 2001). The *DSPP* gene contains five exons encoding the most abundant non-collagenous protein in the dentine. The last two exons of *DSPP* together comprise more than 96 per cent of the coding sequence. Exons 1–4 of the gene encode for DSP while exon 5 encodes for the C-terminus of DSP and the whole DPP protein (Gu, Chang, Ritchie, Clarkson, & Rutherford, 2000). These proteins undergo several post-translational modifications involving glycosylation and phosphorylation to regulate induction and mineralisation of the dentine. Expression of a DSP transgene in the *Dspp* null mice indicated the distinct roles of DSP and DPP in dentinogenesis. DSP regulated the mineralisation initiation, whereas DPP controlled the mineral maturation (Suzuki et al., 2009).

A large number of in-frame indels, non-synonymous single nucleotide polymorphisms (SNPs), and synonymous SNPs were found in the DPP coding region of the normal population. These were shown to be in Hardy–Weinberg equilibrium (Song, Wang, Fan, Su, & Bian, 2008). The indels usually were triplet which resulted in an in-frame insertion or deletion of amino acids and length variations in DPP. The variations that preserve the reading frame or a single missense variant in the DPP-repeated region would therefore be unlikely to cause deleterious effects on protein functions. In addition, the existence of wide-ranging sequence variations in DPP could contribute to the phenotypic variations in human teeth and involve in the evolution of human dentition.

The exon 5 of the *DSPP* gene is composed of more than 200 tandem copies of a nominal 9-bp repeat. This locus is therefore prone to the formation of indels which are likely due to slip-replication events (Maciejewska & Chomik, 2012; Suzuki et al., 2009). The singleton indels are commonly observed in this region. We identified a novel heterozygous frameshift mutation caused by a deletion of four base pairs, c.1915_1918delAAGT (p.K639QfsX674), in exon 5 of the *DSPP* gene. This is located in the DPP coding region which is required for dentine mineralisation. The mutation is expected to convert the reading frame from a tandem hydrophilic and phosphorylated DDS (serine, serine, aspartic acid) repeats to a long range of hydrophobic amino acids rich

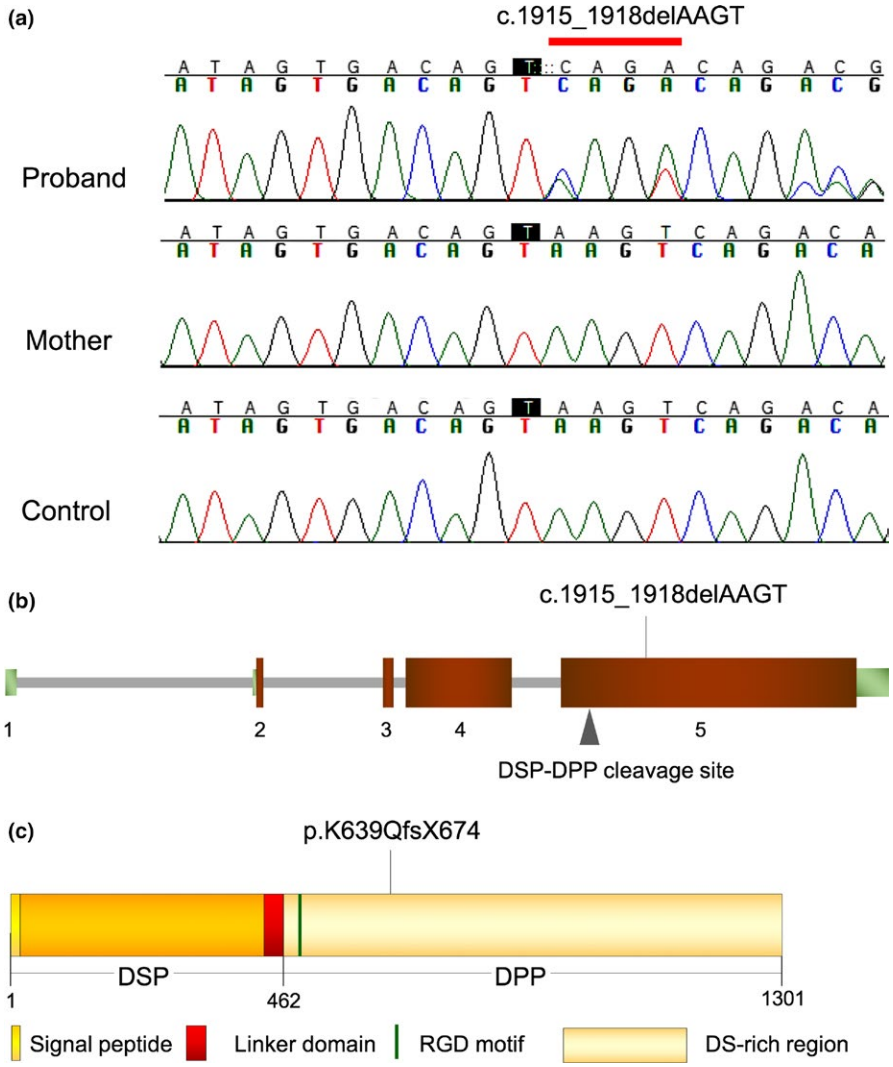

FIGURE 3 Genetic analysis of the proband. (a) An electropherogram demonstrated a novel heterozygous mutation, c.1915_1918delAAGT (p.K639QfsX674), in exon 5 of the dentin sialophosphoprotein (*DSPP*) gene in the proband. The mutation was not observed in his unaffected mother and a control. (b) The mutation was located in exon 5 of the *DSPP* gene. (c) The amino acids were altered in the DSS repeating region of the dentin phosphoprotein (DPP) portion [Colour figure can be viewed at wileyonlinelibrary.com]

in valine, alanine, and isoleucine. These totally change the constitution and arrangement of hydrophilic and acidic variants of DPP protein into hydrophobic amino acids for more than 600 amino acids 3′ to the deletion site. The hydrophobic carboxy-terminal domain extends past the normal stop codon until a new stop codon is reached at 10 codons later.

The phosphorylated form of DDS repeat sequences in the DPP protein is important for calcium binding, aggregation of collagen fibrils, and formation of nucleation of hydroxyapatite (George & Hao, 2005; George et al., 1996). The deficiency of serine in the mutant DPP might result in a decrease in phosphorylated sites and amount of bound calcium interfering with the assembly of calcium phosphate into apatite crystals. Correspondingly, a decrease in mineral density was observed in our DGI dentine. These suggest that the p.K639QfsX674 mutation could disturb the structure and function of DPP in dentine mineralisation. As all the previous reported *DSPP* frameshift mutations in exon 5 resulted in DGI or DD, so here the identified mutation should be responsible for the dental phenotypes in proband. Further functional studies such as immunostaining of DSPP protein will be required to determine the influence of this novel mutation on the protein functions.

Alterations in tooth structures have been inconsistently reported in the permanent teeth which were found in patients with DGI. The unorganised dentinal tubules with the reduction in size and number of the tubules and the decreased hardness were reported in the permanent teeth of DGI patients (Malmgren et al., 2004; Siar, 1986; Song et al., 2006). A previous study showed the irregular appearance of the dentinal tubules in the DGI primary teeth (Davis, Fearne, Sabel, & Noren, 2015). Dentine of *Dspp* null mice exhibited lower elastic modulus and hardness than the wild-type control (Nurrohman et al., 2016). In contrast, *Col1a2*^{oim}mutant mice, which exhibited DGI phenotypes, demonstrated higher hardness and Young's modulus in the dentine as compared with the wild-type mice (Lopez Franco, Huang, Pleshko Camacho, Stone, & Blank, 2006). The data suggested that clinical fragility of mutant teeth was not due to reduced hardness or Young's modulus, but to defects in postyield behaviour or fatigue damage (Lopez Franco et al., 2006). Our proband showed reduced dentine mineral density consistent with previous reports in DGI patients and mice with *Dspp* mutations. These suggest that the mutations in *Dspp* could affect the dentine hardness causing tooth fragility and rapid deterioration of dental hard tissues.

Analysis of mouse *Dspp* revealed three binding sites for *Runx2* (MacDougall et al., 1997). The RUNX2 has been shown to involve in multiple signallings and control the differentiation of dental pulp

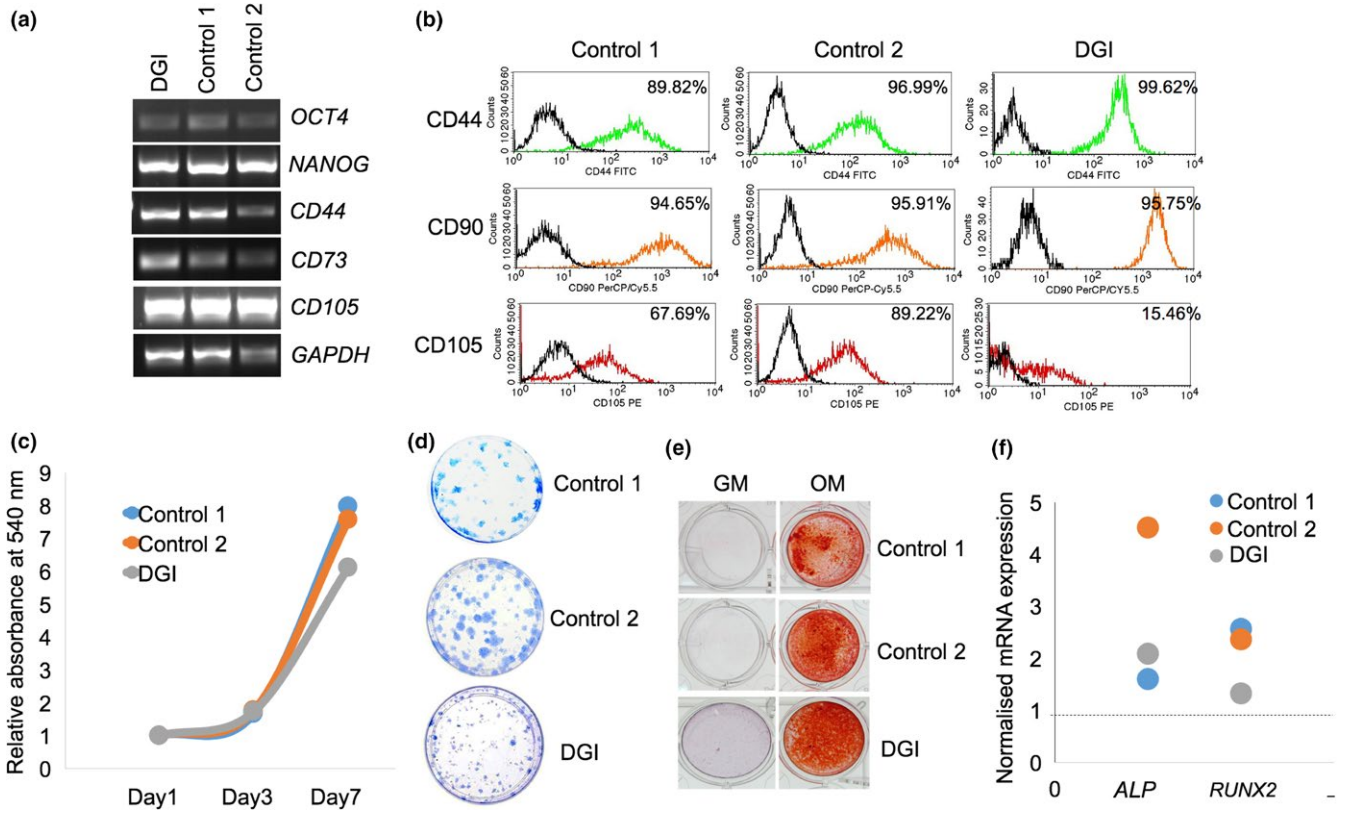

FIGURE 4 Characteristics of cells isolated from dental tissues of the patient with dentinogenesis imperfecta (DGI). (a) The mRNA expression of pluripotent and mesenchymal stem cell markers was examined using semi-quantitative polymerase chain reaction. (b) Surface marker expression was evaluated using flow cytometry analysis. (c) Cell proliferation was determined using the MTT assay at days 1, 3, and 7. (d) Colony formation was stained with Coomassie blue at day 14. (e) Mineral deposition was evaluated by alizarin red S staining at day 14 after induction. (f) *ALP* and *RUNX2* mRNA expressions were examined by real-time quantitative polymerase chain reaction at day 7 after induction. The dotted line indicated the expression of cells cultured in the growth medium [Colour figure can be viewed at wileyonlinelibrary.com]

cells to odontoblasts during dentinogenesis (Yang et al., 2014; Zhang, Chang, Sonoyama, Shi, & Wang, 2008). The expression of *Runx2*, *Gli1*, *Numb*, and *Notch* in the dental pulp cells and odontoblasts was altered in the *Dspp* mutants (Chen et al., 2016). Similarly, DGI cells of our patient showed reduced expression of *RUNX2* in OM media. These lines of evidence suggest that *DSPP* might interact with *RUNX2* in the dental pulp cells to regulate dentine formation and mineralisation.

Although the *DSPP* mutation and lower *RUNX2* expression in pulp cells were observed in our patient, the DGI cells exhibited similar levels of mineralisation to the control. This phenomenon can be explained as follows. First, mineralisation is controlled by several genes, not only *DSPP* and *RUNX2* (Yamada & Giachelli, 2017). Phosphate/pyrophosphate ratio is a key factor in mineralisation process, which can be regulated by various genes including *ALP*, *ANKH*, and *ENPP1* (Foster et al., 2008). Second, although DPP is important to dentine mineralisation, other small integrin-binding ligand N-linked glycoproteins (SIBLINGs) including dentine morphogenetic protein 1 (DMP1), bone sialoprotein (BSP), and osteopontin (OPN) also participate in this process (Foster et al., 2008; Rodrigues et al., 2012). Synchronised function of these proteins influences the dentine mineralisation.

The present study also demonstrated the altered characteristics and behaviours of DGI cells. These cells expressed lower CD105 than the control cells. In addition, the decrease in cell proliferation and colony size was noted while the osteogenic differentiation potency was comparable to the control. In addition to the effects of *DSPP* mutation, the pathological condition of dental pulp may influence the changes of pulp cells; therefore, DGI cells' characteristics should be interpreted with caution. Cells isolated from inflamed dental pulp tissues were previously shown to have changes in inflammatory mediators such as tumour necrotic factor α and toll-like receptors (Fawzy El-Sayed, Klingebiel, & Dörfer, 2016; Yu et al., 2014). Nonetheless, several studies demonstrated no significant differences in cellular behaviours including surface marker expression, proliferation, and differentiation abilities of the cells isolated from inflamed and healthy pulp tissues of both primary and permanent teeth (Böttcher, Scarparo, Batista, Fossati, & Grecca, 2013; Lee, Zhang, Karabucak, & Le, 2016; Tandon, Saha, Rajendran, & Nayak, 2010; Yu et al., 2014). These suggest that infections mainly alter only inflammatory factors of the pulp cells, not cellular behaviours. With the limited numbers of cells from the proband, the potential influences of inflammatory factors on the behaviours of DGI cells could not be investigated in the present study.

CD105 (also known as endoglin) is mainly expressed in endothelial cells. It can also be expressed in other cell types, including vascular smooth muscle cells and fibroblasts (Fonsatti & Maio, 2004).

CD105 is a receptor's component for transforming growth factor-β (Fonsatti & Maio, 2004). It has been introduced as one of the mesenchymal stem cell surface markers (Dominici et al., 2006). CD105 has crucial roles in the anti-apoptosis effect, cell migration, and angiogenesis (Fonsatti & Maio, 2004). In the present study, CD105 mRNA expression was abundant in cells isolated from DGI and the control. However, CD105 protein expression was markedly reduced in cells isolated from the DGI tooth. The infection in DGI pulp tissues may play a role in CD105 reduction as it has been previously reported that an inflammatory cytokine, TNF-α, downregulated CD105 protein, but not mRNA expression in vascular endothelial cells (Li et al., 2003). The role of decreased CD105 in the DGI cells requires further investigations.

In conclusion, we have identified a novel heterozygous frameshift deletion, c.1915_1918delAAGT (p.K639QfsX674), in exon 5 of the *DSPP* gene associated with the defects in dentin mineralisation, collagen arrangement, and formation of dentinal tubules in non-syndromic DGI. This study expands mutation spectrum of the *DSPP* gene and provides an insight into how DGI influences dentinogenesis, pulp cells, and dental treatment leading to the better understanding and proper management of the disease.

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CONFLICT OF INTERESTS

None to declare.

AUTHOR CONTRIBUTIONS

T. Porntaveetus contributed to conception, design, data analysis, drafted and critically revised the manuscript; C. Srichomthong, N. Nowwarote, P. Pavasant contributed to data acquisition and critically revised the manuscript; T. Osathanon, K. Suphapeetiporn contributed to design, data analysis, and critically revised the manuscript; V. Shotelersuk contributed to conception, data analysis, drafted and critically revised the manuscript.

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REFERENCES

Böttcher, D. E., Scarparo, R. K., Batista, E. L., Fossati, A. C. M., & Grecca, F. S. (2013). Histologic evaluation and immunohistochemical localization of STRO-1 and BMP-4 in rat immature teeth: A comparison between vital and induced pulp necrosis. *Archives of Oral Biology*, *58*, 1174–1179. https://doi.org/10.1016/j.archoralbio.2013.04.001

Butler, W. T., & Ritchie, H. (1995). The nature and functional significance of dentin extracellular matrix proteins. *International Journal of Developmental Biology*, *39*, 169–179.

Chen, Y., Zhang, Y., Ramachandran, A., & George, A. (2016). DSPP is essential for normal development of the dental-craniofacial complex. *Journal of Dental Research*, *95*, 302–310. https://doi.org/10.1177/0022034515610768

Davis, G. R., Fearne, J. M., Sabel, N., & Noren, J. G. (2015). Microscopic study of dental hard tissues in primary teeth with Dentinogenesis Imperfecta Type II: Correlation of 3D imaging using X-ray microtomography and polarising microscopy. *Archives of Oral Biology*, *60*, 1013–1020. https://doi.org/10.1016/j.archoralbio.2015.03.010

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., & Horwitz, E. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*, *8*, 315–317. https://doi.org/10.1080/14653240600855905

Fawzy El-Sayed, K. M., Klingebiel, P., & Dörfer, C. E. (2016). Toll-like receptor expression profile of human dental pulp stem/progenitor cells. *Journal of Endodontics*, *42*, 413–417. https://doi.org/10.1016/j.joen.2015.11.014

Fonsatti, E., & Maio, M. (2004). Highlights on endoglin (CD105): From basic findings towards clinical applications in human cancer. *Journal of Translational Medicine*, *2*, 18. https://doi.org/10.1186/1479-5876-2-18

Foster, B. L., Tompkins, K. A., Rutherford, R. B., Zhang, H., Chu, E. Y., Fong, H., & Somerman, M. J. (2008). Phosphate: Known and potential roles during development and regeneration of teeth and supporting structures. *Birth Defects Research Part C: Embryo Today: Reviews*, *84*, 281–314. https://doi.org/10.1002/bdrc.20136

George, A., Bannon, L., Sabsay, B., Dillon, J. W., Malone, J., Veis, A., & Copeland, N. G. (1996). The carboxyl-terminal domain of phosphophoryn contains unique extended triplet amino acid repeat sequences forming ordered carboxyl-phosphate interaction ridges that may be essential in the biomineralization process. *Journal of Biological Chemistry*, *271*, 32869–32873. https://doi.org/10.1074/jbc.271.51.32869

George, A., & Hao, J. (2005). Role of phosphophoryn in dentin mineralization. *Cells Tissues Organs*, *181*, 232–240. https://doi.org/10.1159/000091384

Gronthos, S., Brahim, J., Li, W., Fisher, L. W., Cherman, N., Boyde, A., & Shi, S. (2002). Stem cell properties of human dental pulp stem cells. *Journal of Dental Research*, *81*, 531–535. https://doi.org/10.1177/154405910208100806

Gronthos, S., Mankani, M., Brahim, J., Robey, P. G., & Shi, S. (2000). Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proceedings of the National Academy of Sciences U S A*, *97*, 13625–13630. https://doi.org/10.1073/pnas.240309797

Gu, K., Chang, S., Ritchie, H. H., Clarkson, B. H., & Rutherford, R. B. (2000). Molecular cloning of a human dentin sialophosphoprotein gene. *European Journal of Oral Sciences*, *108*, 35–42. https://doi.org/10.1034/j.1600-0722.2000.00765.x

Huang, B., Sun, Y., Maciejewska, I., Qin, D., Peng, T., McIntyre, B., & Qin, C. (2008). Distribution of SIBLING proteins in the organic and inorganic phases of rat dentin and bone. *European Journal of Oral Sciences*, *116*, 104–112. https://doi.org/10.1111/j.1600-0722.2008.00522.x

Kim, J. W., & Simmer, J. P. (2007). Hereditary dentin defects. *Journal of Dental Research*, *86*, 392–399. https://doi.org/10.1177/154405910708600502

Lee, S., Zhang, Q. Z., Karabucak, B., & Le, A. D. (2016). DPSCs from inflamed pulp modulate macrophage function via the TNF-alpha/IDO axis. *Journal of Dental Research*, *95*, 1274–1281. https://doi.org/10.1177/0022034516657817

Levin, L. S., Leaf, S. H., Jelmini, R. J., Rose, J. J., & Rosenbaum, K. N. (1983). Dentinogenesis imperfecta in the Brandywine isolate (DI type III): Clinical, radiologic, and scanning electron microscopic studies of the dentition. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *56*, 267–274. https://doi.org/10.1016/0030-4220(83)90008-7

Li, C., Guo, B., Ding, S., Rius, C., Langa, C., Kumar, P., & Kumar, S. (2003). TNF alpha down-regulates CD105 expression in vascular endothelial cells: A comparative study with TGF beta 1. *Anticancer Research*, *23*, 1189–1196.

Lopez Franco, G. E., Huang, A., Pleshko Camacho, N., Stone, D. S., & Blank, R. D. (2006). Increased Young's modulus and hardness of Col1a2oim dentin. *Journal of Dental Research*, *85*, 1032–1036. https://doi.org/10.1177/154405910608501111

MacDougall, M., Simmons, D., Luan, X., Nydegger, J., Feng, J., & Gu, T. T. (1997). Dentin phosphoprotein and dentin sialoprotein are cleavage products expressed from a single transcript coded by a gene on human chromosome 4: Dentin phosphoprotein DNA sequence determination. *Journal of Biological Chemistry*, *272*, 835–842. https://doi.org/10.1074/jbc.272.2.835

Maciejewska, I., & Chomik, E. (2012). Hereditary dentine diseases resulting from mutations in DSPP gene. *Journal of Dentistry*, *40*, 542–548. https://doi.org/10.1016/j.jdent.2012.04.004

Malmgren, B., Lindskog, S., Elgadi, A., & Norgren, S. (2004). Clinical, histopathologic, and genetic investigation in two large families with dentinogenesis imperfecta type II. *Human Genetics*, *114*, 491–498. https://doi.org/10.1007/s00439-004-1084-z

Nowwarote, N., Pavasant, P., & Osathanon, T. (2015). Role of endogenous basic fibroblast growth factor in stem cells isolated from human exfoliated deciduous teeth. *Archives of Oral Biology*, *60*, 408–415. https://doi.org/10.1016/j.archoralbio.2014.11.017

Nowwarote, N., Sukarawan, W., Pavasant, P., & Osathanon, T. (2017). Basic fibroblast growth factor regulates REX1 expression via IL-6 in stem cells isolated from human exfoliated deciduous teeth. *Journal of Cellular Biochemistry*, *118*, 1480–1488. https://doi.org/10.1002/jcb.25807

Nurrohman, H., Saeki, K., Carneiro, K., Chien, Y. C., Djomehri, S., Ho, S. P., & Habelitz, S. (2016). Repair of dentin defects from DSPP knockout mice by PILP mineralization. *Journal of Material Research*, *31*, 321–327. https://doi.org/10.1557/jmr.2015.406

O'Connell, A. C., & Marini, J. C. (1999). Evaluation of oral problems in an osteogenesis imperfecta population. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *87*, 189–196. https://doi.org/10.1016/S1079-2104(99)70272-6

Porntaveetus, T., Srichomthong, C., Ohazama, A., Suphapeetiporn, K., & Shotelersuk, V. (2017). A novel GJA1 mutation in oculodentodigital dysplasia with extensive loss of enamel. *Oral Diseases*, *23*, 795-800.https://doi.org/10.1111/odi.12663

Rodrigues, T. L., Foster, B. L., Silverio, K. G., Martins, L., Casati, M. Z., Sallum, E. A., & Nociti Jr, F. H. (2012). Hypophosphatasia-associated deficiencies in mineralization and gene expression in cultured dental pulp cells obtained from human teeth. *Journal of Endodontics*, *38*, 907–912. https://doi.org/10.1016/j.joen.2012.02.008

Siar, C. H. (1986). Quantitative histological analysis of the human coronal dentine in dentinogenesis imperfecta types I and II. *Archives of Oral Biology*, *31*, 387–390. https://doi.org/10.1016/0003-9969(86)90162-7

Song, Y. L., Wang, C. N., Fan, M. W., Su, B., & Bian, Z. (2008). Dentin phosphoprotein frameshift mutations in hereditary dentin disorders and their variation patterns in normal human population. *Journal of Medical Genetics*, *45*, 457–464. https://doi.org/10.1136/jmg.2007.056911

Song, Y., Wang, C., Peng, B., Ye, X., Zhao, G., Fan, M., & Bian, Z. (2006). Phenotypes and genotypes in 2 DGI families with different DSPP mutations. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *102*, 360–374. https://doi.org/10.1016/j.tripleo.2005.06.020

Sreenath, T. L., Cho, A., MacDougall, M., & Kulkarni, A. B. (1999). Spatial and temporal activity of the dentin sialophosphoprotein gene promoter: Differential regulation in odontoblasts and ameloblasts. *International Journal of Developmental Biology*, *43*, 509–516.

Sreenath, T., Thyagarajan, T., Hall, B., Longenecker, G., D'Souza, R., Hong, S., & Kulkarni, A. B. (2003). Dentin sialophosphoprotein knockout mouse teeth display widened predentin zone and develop defective dentin mineralization similar to human dentinogenesis imperfecta type III. *Journal of Biological Chemistry*, *278*, 24874–24880. https://doi.org/10.1074/jbc.M303908200

Suzuki, S., Sreenath, T., Haruyama, N., Honeycutt, C., Terse, A., Cho, A., & Kulkarni, A. B. (2009). Dentin sialoprotein and dentin phosphoprotein have distinct roles in dentin mineralization. *Matrix Biology*, *28*, 221–229. https://doi.org/10.1016/j.matbio.2009.03.006

Tandon, S., Saha, R., Rajendran, R., & Nayak, R. (2010). Dental pulp stem cells from primary and permanent teeth: Quality analysis. *Journal of Clinical Pediatric Dentistry*, *35*, 53–58. https://doi.org/10.17796/jcpd.35.1.e33x0135r7670287

Xiao, S., Yu, C., Chou, X., Yuan, W., Wang, Y., Bu, L., & Kong, X. (2001). Dentinogenesis imperfecta 1 with or without progressive hearing loss is associated with distinct mutations in DSPP. *Nature Genetics*, *27*, 201–204. https://doi.org/10.1038/84848

Yamada, S., & Giachelli, C. M. (2017). Vascular calcification in CKD-MBD: Roles for phosphate, FGF23, and Klotho. *Bone*, *100*, 87–93. https://doi.org/10.1016/j.bone.2016.11.012

Yang, F., Xu, N., Li, D., Guan, L., He, Y., Zhang, Y., & Zhang, X. (2014). A feedback loop between RUNX2 and the E3 ligase SMURF1 in regulation of differentiation of human dental pulp stem cells. *Journal of Endodoctics*, *40*, 1579–1586. https://doi.org/10.1016/j.joen.2014.04.010

Yu, S., Diao, S., Wang, J., Ding, G., Yang, D., & Fan, Z. (2014). Comparative analysis of proliferation and differentiation potentials of stem cells from inflamed pulp of deciduous teeth and stem cells from exfoliated deciduous teeth. *BioMed Research International*, *2014*, 930907.

Zhang, C., Chang, J., Sonoyama, W., Shi, S., & Wang, C. Y. (2008). Inhibition of human dental pulp stem cell differentiation by Notch signaling. *Journal of Dental Research*, *87*, 250–255. https://doi.org/10.1177/154405910808700312

Zhang, X., Zhao, J., Li, C., Gao, S., Qiu, C., Liu, P., & Shen, Y. (2001). DSPP mutation in dentinogenesis imperfecta Shields type II. *Nature Genetics*, *27*, 151–152. https://doi.org/10.1038/84765

SUPPORTING INFORMATION

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