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Novel dentin sialophosphoprotein gene frameshift mutations affect dentin mineralization

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ABSTRACT

Objective: This study aimed to identify candidate genes for inheritable dentin defects in three Chinese pedigrees and characterize the property of affected teeth.
Design: Clinical and radiological features were recorded for the affected individuals. Genomic DNA obtained from peripheral venous blood or saliva were analyzed by whole-exome sequencing. The density and microhardness of affected dentin was measured. Scanning electron microscopy (SEM) was also performed to obtain the microstructure phenotype.
Results: 1) General appearance: the affected dentitions shared yellowish-brown or milky color. Radiographs showed that the pulp cavity and root canals were obliterated in varying degrees or exhibited a pulp aspect in the 'thistle tube'. Some patients exhibited periapical infections without pulpal exposure, and some affected individuals showed shortened, abnormally thin roots accompanied by severe alveolar bone loss. 2) Genomic analysis: three new frameshift mutations (NM_014208.3: c.2833delA, c.2852delGand c.3239delA) were identified in exon 5 of dentin sialophosphoprotein (*DSPP*) gene, altering dentin phosphoprotein (DPP) as result. In vitro studies showed that the density and microhardness of affected dentin were decreased, the dentinal tubules were sparse and arranged disorderly, and the dentinal-enamel-junction (DEJ) was abnormal.
Conclusions: In this study, we identified three novel frameshift mutations of dentin sialophosphoprotein gene related to inherited dentin defects. These mutations are speculated to cause abnormal coding of dentin phosphoprotein C-terminus, which affect dentin mineralization. These results expand the spectrum of dentin sialophosphoprotein gene mutations causing inheritable dentin defects and broaden our understanding of the biological mechanisms by which dentin forms.

1. Introduction

Inherited dentin malformations are autosomal dominant disorders of dentin mineralization and are separated into two main categories, dentinogenesis imperfecta (DGI) and dentin dysplasia (DD) (Shields et al., 1973). Mutations in dentin sialophosphoprotein (*DSPP)* gene are clinically related to dentinogenesis imperfecta (types II and III) and dentin dysplasia (type II) (De La Dure-Molla et al., 2015; Kim & Simmer, 2007). To date, 60 different dentin sialophosphoprotein gene mutations have been reported to cause inherited dentin defects in human. Clinically, it is characterized by amber and opalescent teeth, poorly mineralized dentin, fractured dental enamel and early obliteration of the pulp chamber (Acevedo et al., 2008; Zhang et al., 2001). Dentin dysplasia type II is similar to dentinogenesis imperfecta type II in terms of deciduous dentition. The affected permanent teeth display mild discoloration, radiographically thistle-shaped pulp chambers and pulp stones (Brenneise & Conway, 1999).

Studies have shown that dentin sialophosphoprotein is mainly

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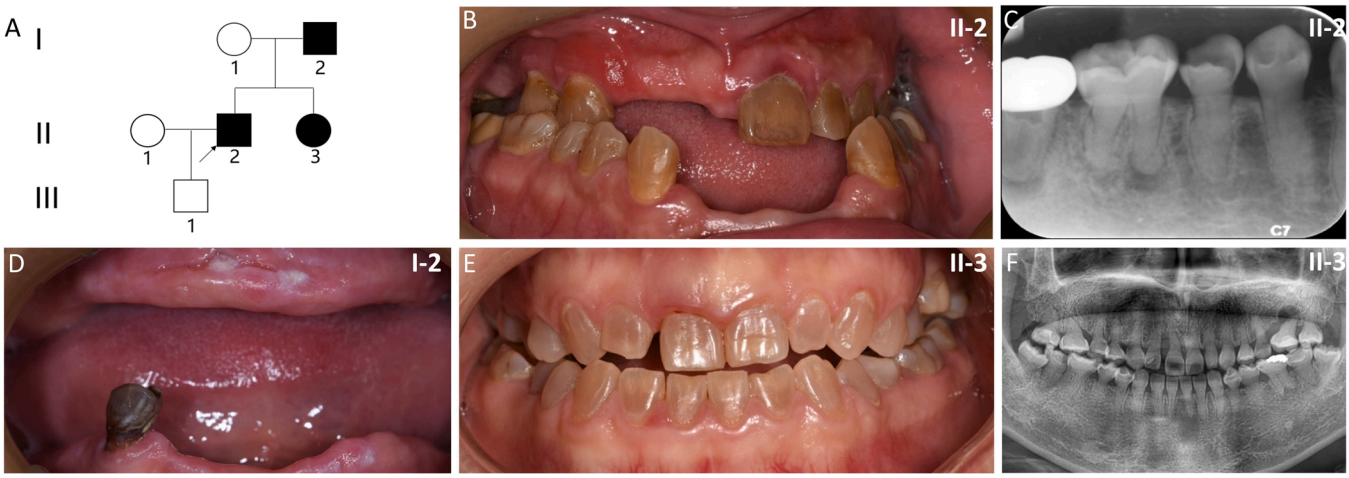

Fig. 1. Pedigree tree and clinical images of family 1. (A): Pedigree tree of family 1; (B, D, and E): Intraoral photographs of proband (II-2), I-2, and II-3; (C): Periapical photograph of II-2; (F): Panoramic photograph of II-3. Arrow indicates the proband.

expressed in odontoblasts and transiently expressed in preameloblasts during tooth development (Chen et al., 2009). The N-terminus of the dentin sialophosphoprotein is cleaved into glycosylated dentin sialoprotein (DSP) and dentin glycoprotein (DGP). The C-terminal region, dentin phosphoprotein (DPP), is aspartic acid and phosphoserine-rich with a unique sequence of serine-serine-asparagine/aspartate (Ser--Ser-Asx) repeats (Song et al., 2008). Dentin phosphoprotein is more abundant and has been suggested to be involved in initiating dentin matrix mineralization (MacDougall et al., 1997; Suzuki et al., 2009). Dentin sialophosphoprotein gene mutations cause inherited dentin defect through a dominant-negative mechanism (Von Marschall et al., 2012). Deletion or point mutations near the signal peptide might affect signal cleavage and interrupt protein folding and secretion, inducing cell pathology and endoplasmic reticulum (ER) stress (Nieminen et al., 2011). The pathological mechanisms might be better described as a gain-of-function or dominant-negative effect (Von Marschall et al., 2012; Nieminen et al., 2011; Shi et al., 2020). However, the molecular genetic etiology and genotype–phenotype correlations remain unclear.

In the current study, we characterized the dental phenotype and the results of a mutational analysis of three Chinese families with clinically identified inherited dentin diseases. All the mutations were in dentin phosphoprotein coding regions, and the differential phenotypes and the characteristics of affected teeth were investigated.

2. Materials and methods

The project was ethically reviewed and approved by the Ethical Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-202278104) and was performed in accordance with the Declaration of Helsinki principles. Written informed consent was obtained from all participants. Three pedigrees were recruited for this study. The probands of the three families sought medical advice for dental caries. Clinical and radiological features were obtained from the affected individuals.

2.1. DNA extraction

Peripheral venous blood from the probands were collected to extract the genomic DNA using a DNA TIANamp Blood DNA mini kit (TIANGEN, Beijing, China) following the manufacturer's instructions. Genomic DNA of other family members were extracted from saliva using a BeaverBeads™ Saliva DNA Kit (BEAVER, Suzhou, China).

2.2. Whole-exome sequencing

Genomic DNA samples of the probands were sent to Euler Genomics (Euler Genomics, Beijing, China) for Whole-exome sequencing (WES). Sequencing was conducted on an Illumina sequencing platform (Illumina, San Diego, CA, USA). To obtain the candidate causative genes, the benign or suspected benign mutations and synonymous mutations were excluded from the gene lists. Next, single nucleotide variants (SNVs) and insertions/deletions (InDels) with a minor allele frequency (MAF) > 0.01 in bioinformatics databases, including the 1000 Genomes Project data in Ensembl (http://asia.ensembl.org/Homo_sapiens/Info/Index), the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org), the single nucleotide polymorphism database (dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi), and the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org) were excluded. Then, candidate genes associated with developmental dental defect were analyzed. Finally, the pathogenicity of the remaining gene mutations was predicted using Sorting Intolerant From Tolerant (SIFT, http://sift.jcvi.org/) and polymorphism phenotyping (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/). Dentin sialophosphoprotein gene, the most common causative gene related to dentinogenesis imperfecta type II, III, and dentin dysplasia type II, were observed in all the participants in our study. The mutations were predicted to be pathogenic or likely pathogenic. Collagen type1 (COL1A1) gene mutations, which are associated with dentinogenesis imperfecta type I, could not be detected in the list. The identified variants were validated for their segregation within each family by WES.

2.3. Microcomputerized tomography

A maxillary second premolar due to severe tooth defect and mobility from II-3 in family 1 was collected, and four control premolars were collected from the healthy patients in similar age for orthodontic reasons. The teeth were fixed in 4% paraformaldehyde fix solution (PFA, Beyotime, Beijing, China) at 4 ℃ for 16 h and then scanned by microcomputerized tomography (μCT, Siemens, Berlin, Germany). Images were reconstructed, and mineral density was measured by Mimics 20 (Materialise, Brussels, Belgium). A volume of 0.027 mm^3 was used for density measurement. Each measurement was repeated 5 times.

2.4. Microhardness

After μCT scanning, the extracted premolar of II-3 in family 1 and normal control premolars were embedded in acrylic resin and cut buccolingually at the midline using a rotary diamond saw. The specimens

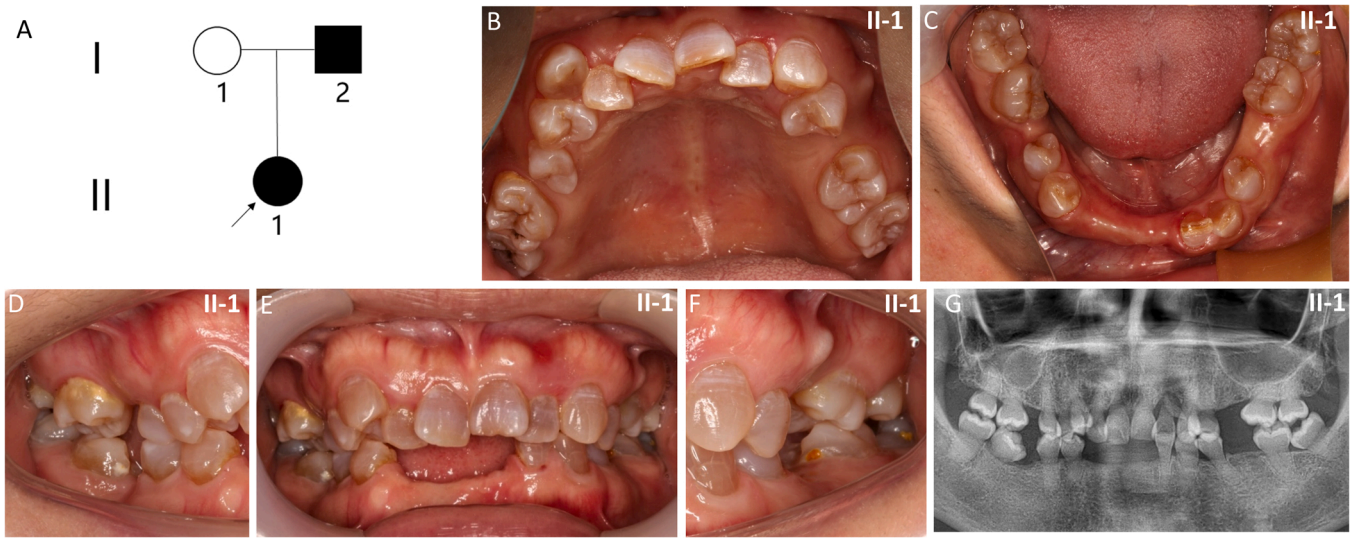

Fig. 2. Pedigree tree and clinical images of family 2. (A): Pedigree tree of family 2; (B-F): Intraoral photographs of the proband (II-1); (G): Panoramic photograph of II-1. Arrow indicates the proband.

were then polished with a series of SIC papers (320, 500, 800 and 1000 grit). The microhardness of each point was measured five times and averaged using a microhardness tester (Shimadzu, Tokyo, Japan). A 0.490 N load was conducted for 15 s to obtain the measurement.

2.5. Scanning electron microscopy

The isolated maxillary first molar of II-2 in family 1 was collected due to severe periodontitis, and the four normal control molars was collected for same reason. After sectioning buccolingually, the specimens were etched with 37% phosphoric acid for 30 s and washed with double distilled water for 30 s to remove the smear layer. Graded ethanol and vacuum-drying were performed to dehydrate the samples. Samples were sputter-coated with gold after vacuum-drying and observed by scanning electron microscopy (SEM, Hitachi, Tokyo, Japan). Images were obtained at 5.0 kV.

2.6. Statistical analysis

ANOVA was performed to analyze the data by using SPSS 24.0 (IBM Corp., Armonk, NY, USA), and each measurement was repeated at least 3 times. Results were represented as mean ± SD, and were visualized by GraphPad Prism 9 (GraphPad Prism, San Diego, CA, USA). *P*-value < 0.05 was considered statistically significant.

3. Results

3.1. Clinical and radiographic manifestations

Based on Shileds' classification in 1973, the yellowish-brown or opalescent tooth color, obliterated or partially obliterated pulp cavity and root canals or 'thistle tube' by X-ray indicate typical clinical manifestations of dentinogenesis imperfecta or dentin dysplasia (Shields et al., 1973). The proband in family 1 was a 35-yr-old male patient from a nonconsanguineous Chinese family, and the inheritance mode in family 1 was autosomal dominant pattern (Fig. 1A). The affected

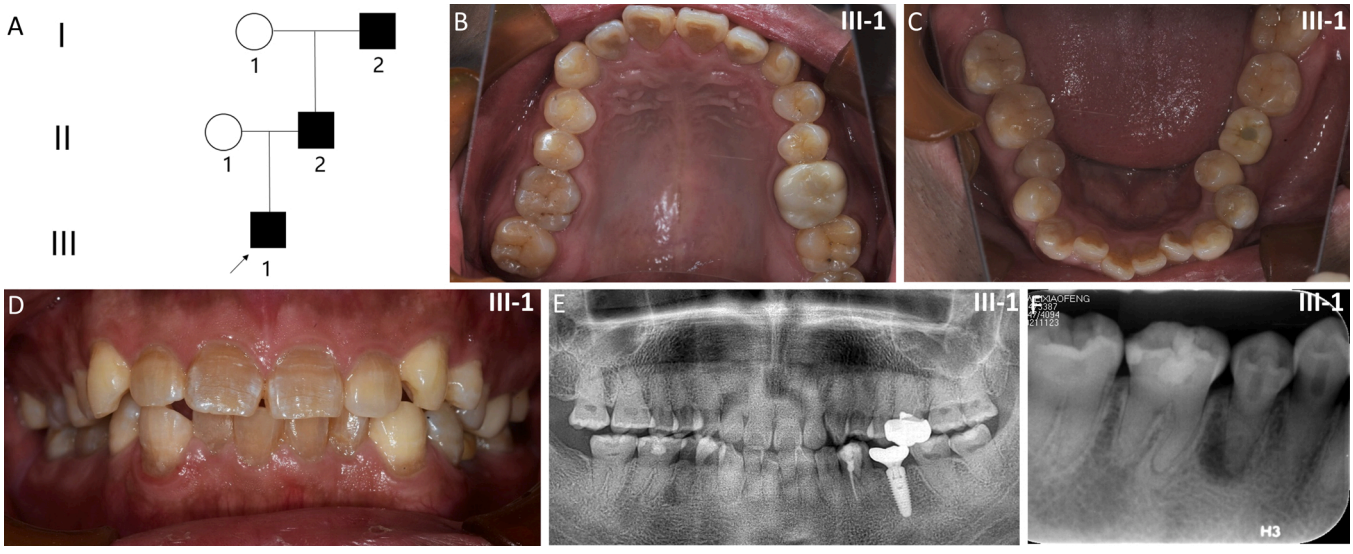

Fig. 3. Pedigree tree and clinical images of family 3. (A): Pedigree tree of family 3; (B-D): Intraoral photographs of the proband (III-1); (E): Panoramic photograph of III-1. (F): Periapical photograph of III-1. Arrow indicates the proband.

Table 1
Summary of mutations in dentin sialophosphoprotein (DSPP) gene causing inherited dentin defects. bp: base pair. del: delete. ins: insert. dup: duplicate.

Location	cDNA	Diagnosis	Reference	Number
Exon2	c 0.16 T > G	DD-II	(Rajpar et al., 2002)	5
	c 0.44 C>T	DGI-II	(Malmgren et al., 2004)	
	c 0.49 C>A	DGI-I	(Xiao et al., 2001)	
	c 0.49 C>T	DGI-II	(Zhang et al., 2007)	
	c 0.50 C>T	DGI-II	(Wang et al., 2011)	
Intron2	c.52–6 T > G	DD-II	(Lee et al., 2009)	7
	c.52–1 G>A	DGI-II	(Liu et al., 2016)	
	c.52–3 C>G	DGI-II	(Kim et al., 2004)	
	c.52–3 C>A	DGI-II	(Holappa et al., 2006)	
	c.52–25_3del	DGI-II	(Wang et al., 2009)	
	c.52–2 A>G	DGI-III	(Li et al., 2017)	
	c.52–2Adel	DGI-II	(Kim et al., 2022)	
Exon3	c 0.52 G>T	DGI-I	(Xiao et al., 2001)	5
	c 0.53 T > A	DGI-II	(Lee et al., 2009)	
	c 0.53 T > C	DGI-III	(Simmer et al., 2022)	
	c 0.133 C>T	DGI-II	(Zhang et al., 2001)	
	c 0.135 G>A	DD-II	(Kim et al., 2022)	
Intron3	c.135 + 1 G>A	DGI-II	(Xiao et al., 2001)	5
	c.135 + 1 G>C	DGI-III	(Kim et al., 2022)	
	c.135 + 1 G>T	DGI-II	(McKnight et al., 2008a)	
	c.135 + 2 T > C	DGI-II	(Zhang et al., 2011)	
	c.135 + 3 A>G	DGI-II	(Bai et al., 2010)	
Exon4	c.202 A>T	DGI-II	(Malmgren et al., 2004)	2
	c 0.727 G>A	DGI-II	(Li et al., 2012)	
Exon5	c.1686delT	DD-II	(Nieminen et al., 2011)	36
	c.1830delC	DD-II	(Nieminen et al., 2011)	
	c.1918_1921delTCAG	DD-II	(Nieminen et al., 2011)	
	c.1922_1925delACAG	DD-II	(Nieminen et al., 2011)	
	c.2063delA	DD-II	(Nieminen et al., 2011)	
	c.2349delT	DGI-II	(Nieminen et al., 2011)	
	c.2666delG	DGI-II	(Nieminen et al., 2011)	
	c.3582_3591del10 bp	DGI-II	(Nieminen et al., 2011)	
	c.3625_3700del76 bp	DGI-II	(Nieminen et al., 2011)	
	c.1870_1873delTCAG	DD-II	(McKnight et al., 2008a)	
	c.1918_1921delTCAG	DD-II	(McKnight et al., 2008a)	
	c.2272delA	DGI-II	(McKnight et al., 2008a)	
	c.2525delG	DGI-II	(McKnight et al., 2008a)	
	c.3141delC	DD-II	(McKnight et al., 2008b)	
	c.3509_3521del13 bp	DGI-II	(Li et al., 2017)	
	c.1874_1877delACAG	DD-II	(Li et al., 2017)	
	c.1915_1918delAAGT	DGI-II	(Porntaveetus et al., 2018)	
	c.2035delA	DD-II	(Yuan et al., 2022)	
	c.2040delC	DD-II	(Song et al., 2008)	
	c.2593delA	DGI-II	(Song et al., 2008)	
	c.2684delG	DGI-II	(Song et al., 2008)	
	c.3438delC	DGI-II	(Song et al., 2008)	
	c.3546_3550delTAGCAinsG	DGI-II	(Song et al., 2008)	
	c.2688delT	DGI-II	(Lee et al., 2011)	
	c.3560delG	DGI-II	(Lee et al., 2011)	
	c.3682_3686del	DGI	(Prasad et al., 2015)	
	c.3480del	DD	(Prasad et al., 2015)	
	c.3676del	DGI	(Bloch-Zupan et al., 2016)	
	c.3504_3508dup	DGI-II	(Yang et al., 2016)	
	c.3533_3534insTA	DGI	(Prasad et al., 2015)	
	36 bp (3599–3634)del, 18 bp (3715–3716)ins	DGI-III	(Dong et al., 2005)	
	c.3461delG	DGI-II	(Simmer et al., 2022)	
	c.3700delA	DGI-II/ DD-II?	(Simmer et al., 2022)	
	c.2833delA	DGI-II	This article	
	c.2852delG	DGI-II	This article	
	c.3239delA	DD-II	This article	

dentitions in family 1 shared common appearance with a yellowish-brown or milky color. The enamel was slightly affected due to attrition (Fig. 1B, D, and E). Radiographic examination showed that the pulp cavity and root canals were obliterated in varying degrees (Fig. 1F). Interestingly, the proband exhibited periapical infections without pulpal exposure (Fig. 1C). Similarly, the proband of family 2, a 33-yr-old female patient, showed an opalescent crown and abnormally thin root accompanied by severe alveolar bone loss (Fig. 2G). The proband of family 3 is a 24-yr-old male patient. He showed slight discoloration with normal shaped teeth (Fig. 3B, C, and D). The radiographic examination showed that the pulp cavity and root canals exhibited a pulp aspect in the 'thistle tube' (Fig. 3F). Syndromic diseases, such as osteogenesis imperfecta, could not be observed in all family members. Taken together, the probands of family 1 and 2 were diagnosed as dentinogenesis imperfecta type II, and the proband of family 3 was diagnosed as dentin dysplasia type II.

3.2. Identification of three novel mutations in the dentin sialophosphoprotein gene

WES was performed to identify the potential candidate genes; 9–24 Gb of exome sequence data were obtained, and the average sequencing depth was 80.453–193.876-fold. Ninety-five percent of exonic regions were covered over 20-fold, and the mapping rate exceeded 99.5% (Supplemental Table 1). All of the above informations indicated that the sequencing data were of high quality and quantity and were suitable for further analysis. Overall, among the all known dentin sialophosphoprotein gene mutations causing dentinogenesis imperfecta or dentin dysplasia (Table 1), three novel frameshift mutations (NM_014208.3: c.2833delA, c.2852delG, and c.3239delA, respectively) in exon 5 of dentin sialophosphoprotein gene were revealed (Fig. 4, Supplemental Figs. 1–3). The identified variants have been submitted to Clinvar (SUB13058397) and the sequencing data have been submitted to Sequence Read Archive (SRA, PRJNA954345). According to ACMG (American College of Medical Genetics and Genomics) guideline, the mutations of family 1 and family 2 were predicted to be LP (likely pathogenic), and the mutation of family 3 was predicted to be PAT (pathogenic).

3.3. Physical and morphologic characteristics of affected teeth

Compared with the normal control enamel, the mineral density of

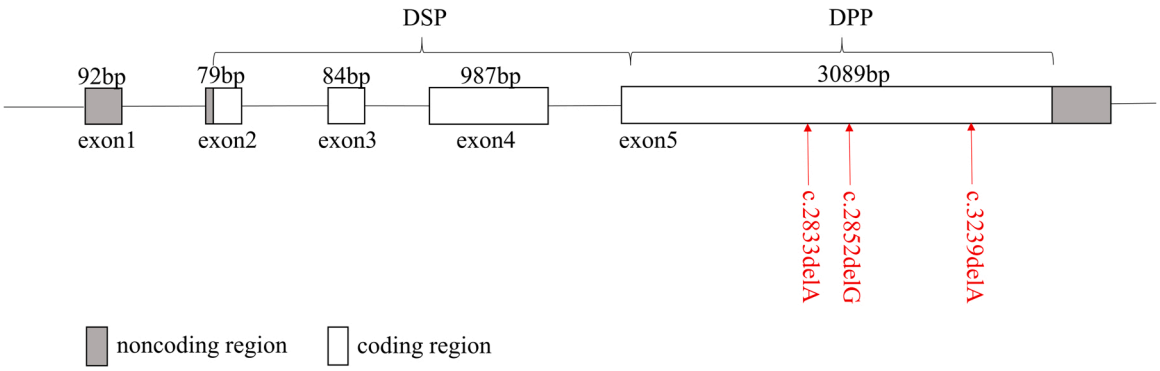

Fig. 4. Identification and diagram of the mutation sites in dentin sialophosphoprotein gene. The exon noncoding region are shaded. The red arrows indicate the position of the mutation sites. bp: base pair.

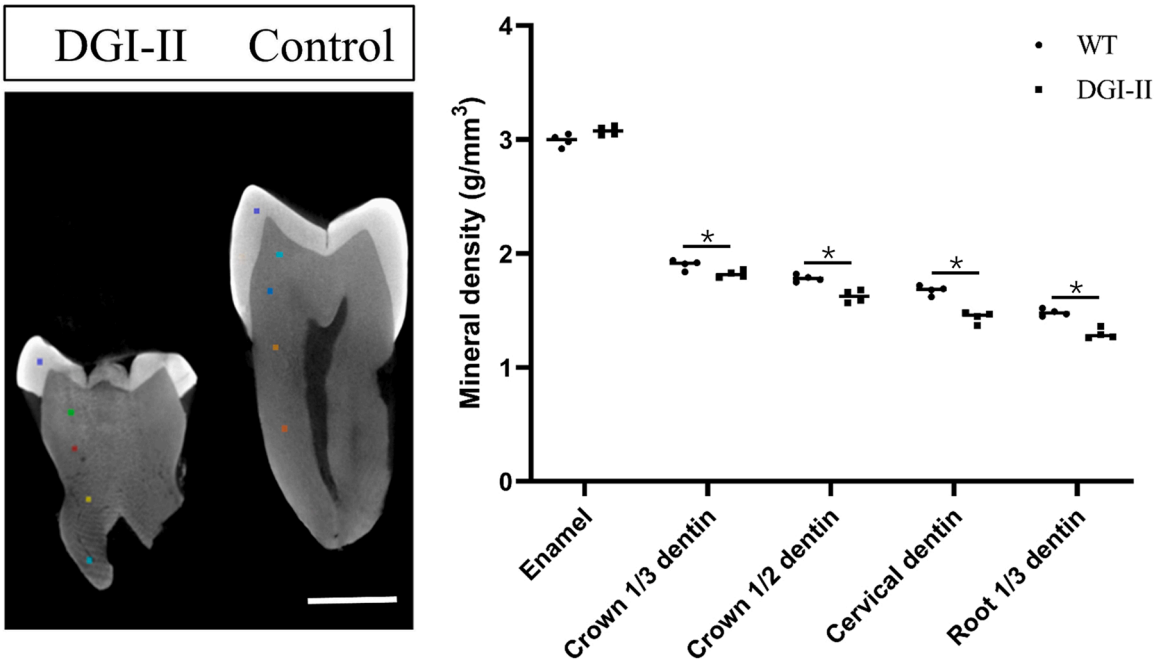

Fig. 5. μCT and mineral density histogram of isolated teeth from II-3 in family 1 and the normal control (ANOVA). n = 4, scale bars: 500 μm.* $p < 0.05$.

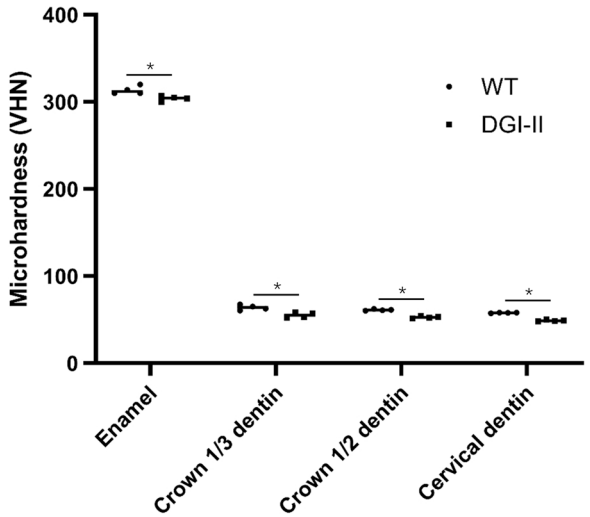

Fig. 6. Microhardness histogram of isolated teeth from II-3 in family 1 and the normal control (ANOVA). n = 4, * $p < 0.05$.

the affected enamel was similar (3.05 vs 2.99 g/cm^3). However, the density of the affected dentin was lower than normal control, especially in the cervical dentin (1.44 vs. 1.68 g/cm^3) and root 1/3 dentin (1.30 vs. 1.48 g/cm^3, Fig. 5). The microhardness numerical values of the affected enamel (304.0 HV), crown 1/3 dentin (55.2 HV), crown 1/2 dentin (53.2 HV) and cervical dentin (49.0 HV) were lower than that of normal control (313.5, 64.0, 61.2, and 57.8 HV, respectively, Fig. 6). SEM revealed that the dentinal tubules of isolated teeth were sparse with disordered arrangement (Fig. 7E), while no obvious abnormality of enamel structure was observed (Fig. 7G). The dentinal-enamel junction (DEJ) and the cemento-dentinal junction (CDJ) were both abnormal (Fig. 7F, H).

4. Discussion

In this study, we characterized the dental phenotype and identified causative mutations in three families with inheritable dentin defects diagnosed as dentinogenesis imperfecta type II and dentin dysplasia type II, respectively. Clinically, the teeth were discolored and radiographically demonstrated structural defects, such as bulbous crowns and small pulp chambers. Three novel frameshift mutations were observed in the dentin phosphoprotein coding region, which were predicted to cause

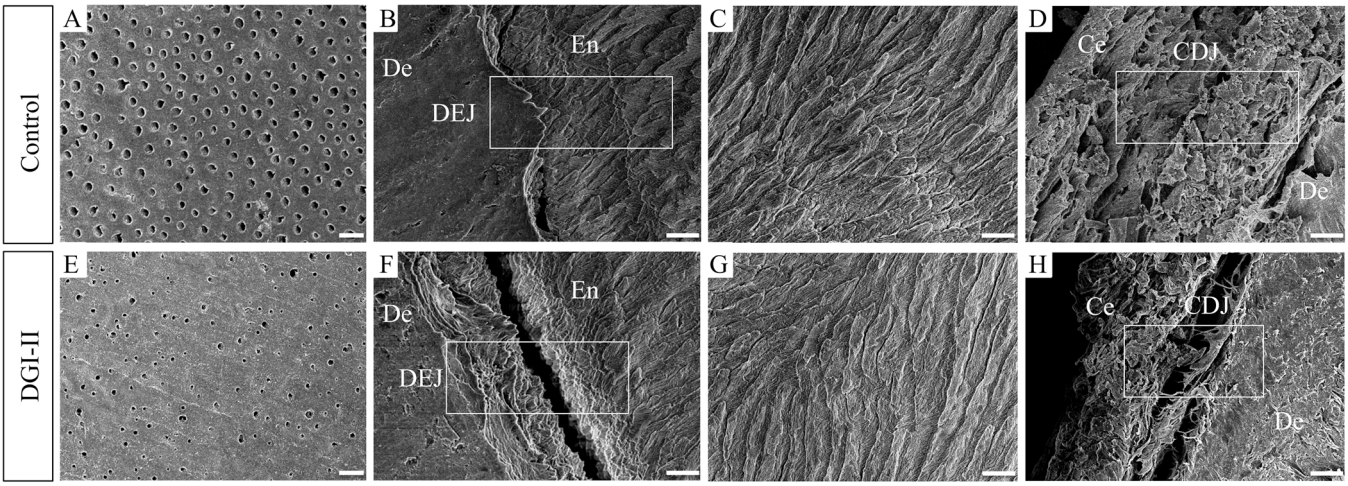

Fig. 7. SEM images of isolated teeth from II-2 in family 1 and the normal control. (A-D): SEM images of normal control; (E-H): SEM images of the isolated tooth from II-2 in family 1. DEJ, dentinal-enamel junction. De, dentin. En, enamel. Ce, cementum. CDJ, cemento-dentinal junction. n = 4, scale bars: 10 μm in A-H.

severe alterations in protein-coding and biochemical properties. The exclusive disease-causing role of the dentin sialophosphoprotein gene mutations in these individuals confirmed the significant role of dentin sialophosphoprotein in dentin mineralization.

Dentin sialophosphoprotein is an aspartic acid and phosphoserine-based protein. It is transiently expressed by preameloblasts in the late bell stage and secreted by odontoblasts throughout the formation of dentin (Yamakoshi, 2008). Dentin sialoprotein and dentin phosphoprotein play different roles in the development of dentin (Liu et al., 2021; Suzuki et al., 2009). It was reported that enamel defects could be observed in some dentinogenesis imperfecta patients, which were only related to mutations of dentin sialoprotein (Simmer et al., 2022; S.-K. Wang et al., 2011). The $Dspp^{P19L/P19L}$ mice, of which the first three amino acids of dentin sialoprotein (isoleucine-proline-valine, IPV) were affected, showed obvious dentin and enamel defects, while the $Dspp^{-1fs/-1fs}$ mice, of which the dentin phosphoprotein was affected, only showed lower dentin mineralization (Liang et al., 2019; Liang, Hu et al., 2021; Liang, Xu et al., 2021). Our study showed that the enamel structure and the mineral density of affected teeth were normal, suggesting that the frameshift mutation of dentin phosphoprotein has no obvious effects on the enamel formation and maturation.

In *Dspp* null mice, the teeth exhibit enlarged pulp chambers, increased predentin zone width, hypomineralization, and pulp exposure. The phenotype is similar to the "shell teeth" observed in dentinogenesis imperfecta type III patients (Sreenath et al., 2003). In dentin phosphoprotein conditional knockout mice, the dentin volume remains normal, but the mineral density of dentin is decreased compared with wild-type control, suggesting a role for dentin phosphoprotein in dentin mineralization and maturation (Suzuki et al., 2009). It was reported that the mineral density and microhardness of affected immature premolar were lower than normal control (Park et al., 2020). Similarly, our study showed that the dentin density and microhardness of affected permanent teeth were decreased. The structures of dentinal-enamel junction and cemento-dentinal junction were both abnormal, and the dentinal tubules were sparse with disordered arrangement, potentially related to the failure of normal dentin maturation due to the dentin phosphoprotein frameshift mutation. Our study showed that the mineralization of cervical dentin and root dentin was more seriously affected than crown dentin, indicating that dentin sialophosphoprotein may play a different role in the development of crown and root. Further studies were needed to investigate the molecular mechanisms in this process.

Comprehensive studies have demonstrated that dentin dysplasia type II and dentinogenesis imperfecta type II can result from mutations in the region encoding dentin phosphoprotein (McKnight et al., 2008a). However, the differential diagnosis between dentin dysplasia type II and dentinogenesis imperfecta type II is challenging. It was believed that N-terminal frameshifts in dentin phosphoprotein were associated with dentin dysplasia type II, and C-terminal frameshifts led to dentinogenesis imperfecta type II (Song et al., 2008). It has been suggested that C-terminal mutations are associated with increased susceptibility to dental complications, such as tooth discoloration and pulpal and root obliteration (Lee et al., 2011; McKnight et al., 2008b; Nieminen et al., 2011). However, in this study, the frameshift mutation in family 3 was located in the C-terminus of dentin phosphoprotein, and the affected individual showed a typical dentin dysplasia type II phenotype with slight discoloration and a thistle-shaped pulp cavity in the permanent teeth, not typical dentinogenesis imperfecta type II phenotype caused by adjacent mutation sites reported in other studies (Song et al., 2008; Nieminen et al., 2011; Li et al., 2017). The mutation location is inconsistent with previous associations between mutation location and dentin dysplasia type II vs. dentinogenesis imperfecta type II. This variability indicates that dentin dysplasia type II and dentinogenesis imperfecta type II are allelic and therefore represent varying degrees of severity of the same disease.

The pathology of the secretory stage could explain why thin dental enamel often undergoes rapid attrition in patients with dentinogenesis imperfecta, which is generally ascribed to a lack of support by the defective underlying dentin (Wang et al., 2011). The susceptibility of teeth to periapical infection without pulp exposure may be explained by defects in enamel and dentin, facilitating the movement of bacteria to the pulp, causing infections (Acevedo et al., 2008). Even without obvious attrition and dentin exposure or alveolar bone loss, periapical radiolucency was observed (Nieminen et al., 2011). Whether dentin sialophosphoprotein is involved in epithelial-mesenchymal interactions during root development, especially in Hertwig's root sheath penetration and cementum and dentin attachment, needs further study.

This study provided a detailed summary and analysis of dentin sialophosphoprotein gene mutations in three families with inherited dentin defects. Current evidence supported the interpretation that dentin sialophosphoprotein gene mutations designations reflected varying levels of dentin defect severity, and the underlying mutations exerted their effects through distinct cellular and molecular mechanisms, suggesting a spectrum of dentin sialophosphoprotein pathological roles in dentinogenesis imperfecta.

CRediT authorship contribution statement

Zhenwei Zhang: Validation, Formal analysis, Investigation, Data

curation, Writing – original draft. **Guibin Huang:** Software, Formal analysis, Investigation, Data curation, Writing – original draft. **Yu Huang:** Formal analysis, Writing – review & editing, Visualization, Funding acquisition. **Siyi Liu:** Methodology, Resources, Writing – review & editing. **Feng Chen:** Methodology, Formal analysis, Writing – review & editing. **Xuejun Gao:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Yanmei Dong:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision, Project administration. **Hua Tian:** Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors have no conflicts of interest relevant to this article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.archoralbio.2023.105701.

References

Acevedo, A. C., Santos, L. J. S., Paula, L. M., Dong, J., & MacDougall, M. (2008). Phenotype characterization and DSPP mutational analysis of three Brazilian dentinogenesis imperfecta type II families. *Cells Tissues Organs, 189*(1–4), 230–236. https://doi.org/10.1159/000152917

Bai, H., Agula, H., Wu, Q., Zhou, W., Sun, Y., Qi, Y., Latu, S., Chen, Y., Mutu, J., & Qiu, C. (2010). A novel DSPP mutation causes dentinogenesis imperfecta type II in a large Mongolian family. *BMC Medical Genetics, 11*(1). https://doi.org/10.1186/1471-2350-11-23

Bloch-Zupan, A., Huckert, M., Stoetzel, C., Meyer, J., Geoffroy, V., Razafindrakoto, R. W., Ralison, S. N., Randrianaivo, J. C., Ralison, G., Andriamasinoro, R. O., Ramanampamaharana, R. H., Randrianazary, S. E., Richard, B., Gorry, P., Manière, M. C., Alson, S. R., & Dollfus, H. (2016). Detection of a novel DSPP mutation by NGS in a population isolate in Madagascar. *Frontiers in Physiology, 7* (MAR), 1–8. https://doi.org/10.3389/fphys.2016.00070

Brenneise, C. V., & Conway, K. R. (1999). Dentin dysplasia, type IIReport of 2 new families and review of the literature. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 87*(6), 752–755. https://doi.org/10.1016/s1079-2104(99)70175-7

Chen, S., Gluhak-Heinrich, J., Wang, Y. H., Wu, Y. M., Chuang, H. H., Chen, L., Yuan, G. H., Dong, J., Gay, I., & MacDougall, M. (2009). Runx2, osx, and dspp in tooth development. *Journal of Dental Research, 88*(10), 904–909. https://doi.org/10.1177/0022034509342873

De La Dure-Molla, M., Philippe Fournier, B., & Berdal, A. (2015). Isolated dentinogenesis imperfecta and dentin dysplasia: Revision of the classification. *European Journal of Human Genetics, 23*(4), 445–451. https://doi.org/10.1038/ejhg.2014.159

Dong, J., Gu, T. T., Jeffords, L., & MacDougall, M. (2005). Dentin phosphoprotein compound mutation in dentin sialophosphoprotein causes dentinogenesis imperfecta type III. *American Journal of Medical Genetics, 132 A*(3), 305–309. https://doi.org/10.1002/ajmg.a.30460

Holappa, H., Nieminen, P., Tolva, L., Lukinmaa, P. L., & Alaluusua, S. (2006). Splicing site mutations in dentin sialophosphoprotein causing dentinogenesis imperfecta type II. *European Journal of Oral Sciences, 114*(5), 381–384. https://doi.org/10.1111/j.1600-0722.2006.00391.x

Kim, J.-W., & Simmer, J. P. (2007). Hereditary Dentin Defects. *Journal of Dental Research, 86*(5), 392–399. ⟨http://www.dentalresearch.org⟩.

Kim, J. W., Nam, S. H., Jang, K. T., Lee, S. H., Kim, C. C., Hahn, S. H., Hu, J. C. C., & Simmer, J. P. (2004). A novel splice acceptor mutation in the DSPP gene causing dentinogenesis imperfecta type II. *Human Genetics, 115*(3), 248–254. https://doi.org/10.1007/s00439-004-1143-5

Lee, K. E., Kang, H. Y., Lee, S. K., Yoo, S. H., Lee, J. C., Hwang, Y. H., Nam, K. H., Kim, J. S., Park, J. C., & Kim, J. W. (2011). Novel dentin phosphoprotein frameshift mutations in dentinogenesis imperfecta type II. *Clinical Genetics, 79*(4), 378–384. https://doi.org/10.1111/j.1399-0004.2010.01483.x

Lee, S. K., Lee, K. E., Jeon, D., Lee, G., Lee, H., Shin, C. U., Jung, Y. J., Lee, S. H., Hahn, S. H., & Kim, J. W. (2009). A novel mutation in the DSPP gene associated with dentinogenesis imperfecta type II. *Journal of Dental Research, 88*(1), 51–55. https://doi.org/10.1177/0022034508328168

Li, D., Du, X., Zhang, R., Shen, B., Huang, Y., Valenzuela, R. K., Wang, B., Zhao, H., Liu, Z., Li, J., Xu, Z., Gao, L., & Ma, J. (2012). Mutation identification of the DSPP in a Chinese family with DGI-II and an up-to-date bioinformatic analysis. *Genomics, 99* (4), 220–226. https://doi.org/10.1016/j.ygeno.2012.01.006

Li, F., Liu, Y., Liu, H., Yang, J., Zhang, F., & Feng, H. (2017). Phenotype and genotype analyses in seven families with dentinogenesis imperfecta or dentin dysplasia. *Oral Diseases, 23*(3), 360–366. https://doi.org/10.1111/odi.12621

Liang, T., Hu, Y., Zhang, H., Xu, Q., Smith, C. E., Zhang, C., Kim, J. W., Wang, S. K., Saunders, T. L., Lu, Y., Hu, J. C. C., & Simmer, J. P. (2021). Mouse Dspp frameshift model of human dentinogenesis imperfecta. *Scientific Reports, 11*(1), 1–14. https://doi.org/10.1038/s41598-021-00219-4

Liang, T., Xu, Q., Zhang, H., Wang, S., Diekwisch, T. G. H., Qin, C., & Lu, Y. (2021). Enamel Defects Associated With Dentin Sialophosphoprotein Mutation in Mice. *Frontiers in Physiology, 12*(September). https://doi.org/10.3389/fphys.2021.724098

Liang, T., Zhang, H., Xu, Q., Wang, S., Qin, C., & Lu, Y. (2019). Mutant Dentin Sialophosphoprotein Causes Dentinogenesis Imperfecta. *Journal of Dental Research, 98*(8), 912–919. https://doi.org/10.1177/0022034519854029

Liu, M. M., Li, W. T., Xia, X. M., Wang, F., Macdougall, M., & Chen, S. (2021). Dentine sialophosphoprotein signal in dentineogenesis and dentine regeneration. *In European Cells and Materials* (Vol. 42). https://doi.org/10.22203/eCM.v042a04

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*(2), 835–842. https://doi.org/10.1074/jbc.272.2.835

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*(5), 491–498. https://doi.org/10.1007/s00439-004-1084-z

McKnight, D. A., Hart, P. S., Hart, T. C., Hartsfield, J. K., Wilson, A., Wright, J. T., & Fisher, L. W. (2008a). A comprehensive analysis of normal variation and disease-causing mutations in the human DSPP gene. *Human Mutation, 29*(12), 1392–1404. https://doi.org/10.1002/humu.20783

McKnight, D. A., Simmer, J. P., Hart, P. S., & Hart, T. C., F. L. W. (2008b). Overlapping DSPP Mutations Cause DD and DGI. *J Dent Res, 87*(12), 1108–1111. ⟨https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf⟩.

Nieminen, P., Papagiannoulis-Lascarides, L., Waltimo-Siren, J., Ollila, P., Karjalainen, S., Arte, S., Veerkamp, J., Tallon Walton, V., Chimenos Küstner, E., Siltanen, T., Holappa, H., Lukinmaa, P. L., & Alaluusua, S. (2011). Frameshift mutations in dentin phosphoprotein and dependence of dentin disease phenotype on mutation location. *Journal of Bone and Mineral Research, 26*(4), 873–880. https://doi.org/10.1002/jbmr.276

Park, H., Hyun, H. K., Woo, K. M., & Kim, J. W. (2020). Physicochemical properties of dentinogenesis imperfecta with a known DSPP mutation. *Archives of Oral Biology, 117*(May), Article 104815. https://doi.org/10.1016/j.archoralbio.2020.104815

Porntaveetus, T., Osathanon, T., Nowwarote, N., Pavasant, P., Srichomthong, C., Suphapeetiporn, K., & Shotelersuk, V. (2018). Dental properties, ultrastructure, and pulp cells associated with a novel DSPP mutation. *Oral Diseases, 24*(4), 619–627. https://doi.org/10.1111/odi.12801

Prasad, M. K., Geoffroy, V., Vicaire, S., Jost, B., Dumas, M., Le Gras, S., Switala, M., Gasse, B., Laugel-Haushalter, V., Paschaki, M., Leheup, B., Droz, D., Dalstein, A., Loing, A., Grollemund, B., Muller-Bolla, M., Lopez-Cazaux, S., Minoux, M., Jung, S., & Bloch-Zupan, A. (2015). A targeted next-generation sequencing assay for the molecular diagnosis of genetic disorders with orodental involvement. *Journal of Medical Genetics, 53*(2), 98–110. https://doi.org/10.1136/jmedgenet-2015-103302

Rajpar, M. H., Koch, M. J., Davies, R. M., Mellody, K. T., Kielty, C. M., & Dixon, M. J. (2002). Mutation of the signal peptide region of the bicistronic gene DSPP affects translocation to the endoplasmic reticulum and results in defective dentine biomineralization. *Human Molecular Genetics, 11*(21), 2559–2565. https://doi.org/10.1093/hmg/11.21.2559

Shi, C., Ma, N., Zhang, W., Ye, J., Shi, H., Xiang, D., Wu, C., Song, L., Zhang, N., & Liu, Q. (2020). Haploinsufficiency of Dspp Gene Causes Dentin Dysplasia Type II in Mice. *Frontiers in Physiology, 11*(November), 1–10. https://doi.org/10.3389/fphys.2020.593626

Shields, E. D., Bixler, D., & El-Kafrawy, A. M. (1973). A proposed classification for heritable human dentine defects with a description of a new entity. *Archives of Oral Biology, 18*(4), 543–553. https://doi.org/10.1016/0003-9969(73)90075-7

Simmer, J. P., Zhang, H., Moon, S. J. H., Donnelly, L. A., Lee, Y., Seymen, F., Koruyucu, M., Chan, H., Lee, K. Y., Wu, S., Hsiang, C., Tsai, A. T. P., Slayton, R. L., Morrow, M., Wang, S., Shields, E. D., & Hu, J. C. (2022). The Modified Shields Classification and 12 Families with Defined DSPP Mutations. *Genes(Basel), 13*(5), 858.

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*(7), 457–464. https://doi.org/10.1136/jmg.2007.056911

Sreenath, T., Thyagarajan, T., Hall, B., Longenecker, G., D'Souza, R., Hong, S., Wright, J. T., MacDougall, M., Sauk, J., & 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*(27), 24874–24880. https://doi.org/10.1074/jbc.M303908200

Suzuki, S., Sreenath, T., Haruyama, N., Honeycutt, C., Cho, A., Kohler, T., Müller, R., Goldberg, M., & Ashok, B. (2009). Dentin sialoprotein and dentin phosphoprotein have distinct roles in dentin mineralization. *Matrix Biology, 28*(4), 221–229. https://doi.org/10.1016/j.matbio.2009.03.006.Dentin

Von Marschall, Z., Mok, S., Phillips, M. D., McKnight, D. A., & Fisher, L. W. (2012). Rough endoplasmic reticulum trafficking errors by different classes of mutant dentin sialophosphoprotein (DSPP) cause dominant negative effects in both dentinogenesis imperfecta and dentin dysplasia by entrapping normal DSPP. *Journal of Bone and Mineral Research, 27*(6), 1309–1321. https://doi.org/10.1002/jbmr.1573

Wang, H. Y., Hou, Y. N., Cui, Y. X., Huang, Y. F., Shi, Y. C., Xia, X. Y., Lu, H. Y., Wang, Y. H., & Li, X. J. (2009). A novel splice site mutation in the dentin sialophosphoprotein gene in a Chinese family with dentinogenesis imperfecta type II. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis, 662*(1–2), 22–27. https://doi.org/10.1016/j.mrfmmm.2008.11.019

Wang, S.-K., Chan, H.-C., Rajderkar, S., Milkovich, R. N., Uston, K. A., Kim, J.-W., Simmer, J. P., & Hu, J. C.-C. (2011). Enamel malformations associated with a defined DSPP mutation in two families. *European Journal Of Oral Sciences, 119*(Suppl 1), 158–167. https://doi.org/10.1111/j.1600-0722.2011.00874.x.Enamel

Xiao, S., Yu, C., Chou, X., Yuan, W., Wang, Y., Bu, L., Fu, G., Qian, M., Yang, J., Shi, Y., Hu, L., Han, B., Wang, Z., Huang, W., Liu, J., Chen, Z., Zhao, G., & Kong, X. (2001). Dentinogenesis imperfecta 1 with or without progressive hearing loss is associated with distinct mutations in DSPP. *Nature Genetics, 27*(2), 201–204. https://doi.org/10.1038/84848

Yamakoshi, Y. (2008). Dentin sialophosphoprotein (DSPP) and dentin. *Journal of Oral Biosciences, 50*(1), 33–44. https://doi.org/10.2330/joralbiosci.50.33

Yang, J., Kawasaki, K., Lee, M., Reid, B. M., Nunez, S. M., Choi, M., Seymen, F., Koruyucu, M., Kasimoglu, Y., Estrella-Yuson, N., Lin, B. P. J., Simmer, J. P., & Hu, J. C. C. (2016). The dentin phosphoprotein repeat region and inherited defects of dentin. *Molecular Genetics and Genomic Medicine, 4*(1), 28–38. https://doi.org/10.1002/mgg3.176

Liu, Yanshan, Huang, Yingzhi, Gao, Jinsong, Li, Shan, & ZhaoX. Z, Xiuli (2016). Identification of a novel mutation of DSPP gene in a Chinese family affected with dentinogenesis imperfecta shields type II. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi, 33*(1), 34–37.

Yuan, M., Zheng, X., Xue, Y., He, Z., Song, G., & Song, Y. (2022). A novel DSPP frameshift mutation causing dentin dysplasia type 2 and disease management strategies. *Oral Diseases, December, 2022*, 1–7. https://doi.org/10.1111/odi.14431

Zhang, J., Wang, J., Ma, Y., Du, W., Zhao, S., Zhang, Z., Zhang, X., Liu, Y., Xiao, H., Wang, H., Jin, L., & Liu, J. (2011). A novel splicing mutation alters DSPP transcription and leads to dentinogenesis imperfecta type II. *PLoS ONE, 6*(11), 4–7. https://doi.org/10.1371/journal.pone.0027982

Zhang, X., Chen, L., Liu, J., Zhao, Z., Qu, E., Wang, X., Chang, W., Xu, C., Wang, Q. K., & Liu, M. (2007). A novel DSPP mutation is associated with type II dentinogenesis Imperfecta in a chinese family. *BMC Medical Genetics, 8*, 1–6. https://doi.org/10.1186/1471-2350-8-52

Zhang, X., Zhao, J., Li, C., Gao, S., Qiu, C., Liu, P., Wu, G., Qiang, B., Lo, W. H. Y., & Shen, Y. (2001). DSPP mutation in dentinogenesis imperfecta shields type II. *Nature Genetics, 27*(2), 151–152. https://doi.org/10.1038/84765