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A Novel Variant in *Dentin Sialophosphoprotein (DSPP)* Gene Causes Dentinogenesis Imperfecta Type III: Case Report

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Keywords: dentinogenesis imperfecta | *DSPP* | hereditary dentin defects | whole exome sequencing

ABSTRACT

Background: Hereditary dentin defects are a group of autosomal dominant disorders characterized by developmental abnormalities in dentin formation and mineralization. They can be categorized into dentin dysplasia and dentinogenesis imperfecta.

Methods: In this study, we report a Chinese family with dentinogenesis imperfecta type III (DGI-III). The proband, a 3-year-old girl, and her mother showed extremely rapid attrition and opalescent discoloration in their teeth. Besides, the primary teeth of the proband showed "shell teeth" radiographically, a phenotype characterized by abnormally enlarged pulp cavities and thin dentin, which are specific features of DGI-III. The clinical data was collected and the genomic DNA was extracted from their peripheral blood samples. Whole-exome sequencing and Sanger sequencing were performed to screen for variations. Then we preliminarily evaluated the secretion of the dentin sialophosphoprotein (DSPP) variant of this family and compared this variant with wild-type DSPP via western blot (WB) analysis in vitro.

Results: The results revealed a novel variant (NM_014208: exon2: c.38C>A: p.A13E) in the signal peptide coding region of the *DSPP* gene in both the proband and her mother, but not in her father, who had normal teeth. The secretion of the variant DSPP protein was not detected in Human embryonic kidney 293E cells via WB analysis.

Conclusion: Taken together, this study describes the clinical features and genetic etiology of a family with DGI-III, expanding the range of variants that cause DGI-III and enriching the phenotypes associated with variants in the signal peptide segment of *DSPP*. Functional analysis reveals that this variant disrupts DSPP protein secretion.

1 | Introduction

Dentin, an important component of the teeth, protects the dental pulp tissue and supports the enamel (Lee et al. 2011). Hereditary dentin defects are a group of autosomal dominant disorders characterized by developmental abnormalities in dentin matrix secretion and mineralization. They are categorized into dentin dysplasia type I (DD-I), DD-II, dentinogenesis imperfecta type I (DGI-I), DGI-II, and DGI-III by Shields et al. (1973). Among them, DD-I (OMIM #125400) is characterized by crescent-shaped pulp, with short roots or rootlessness. DD-II (OMIM #125420) is characterized by "thistle tube pulp" and pulp stones,

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but the roots remain normal. DGI-I (OMIM #166200) refers to dentin development defect combined with osteogenesis imperfecta. In DGI-II (OMIM #125490), both primary and permanent teeth exhibit amber-like translucency and attrition (Su et al. 2023). Meanwhile, the radiographic images show bulbous crowns with cervical constriction, short roots, and obliteration of both pulp and root canals (Su et al. 2023). DGI-III (OMIM #125500) is the least common type of dentinogenesis imperfecta, and has been first observed among "Brandywine isolate", an isolated tri-racial sub-population in Southern Maryland, USA. The prevalence of DGI-III has been closely tied to this particular community (MacDougall et al. 2006). The clinical characteristics of permanent teeth in DGI-III are diverse and similar to those observed in DGI-II. Differently, the primary teeth of DGI-III exhibit multiple pulp exposures due to severe attrition and chipped enamel, and they often manifest "shell teeth" radiographically (Su et al. 2023).

Both collagenous and non-collagenous proteins in the dentin matrix are essential for dentin formation and mineralization. Pathogenic variants in certain genes, including *COL1A1* (MIM* 120150), *COL1A2* (MIM* 120160), *VPS4B* (MIM* 609983), *SSUH2* (MIM* 617479), *SMOC2* (MIM* 607223), and *DSPP* (MIM* 125485), have been identified as causes of hereditary dentin disorders (Su et al. 2023). Several studies have confirmed that variants in *DSPP* are the etiologies of non-syndromic hereditary dentin defects, including DD-II, DGI-II, and DGI-III. To date, all known variants causing DGI-III (c.49C>T, c.50C>T, c.52-2A>G, c.53T>C, c.135+1G>C, compound variants c.3599–3634 del36bp and c.3715–3716 ins18bp) are located on the *DSPP* gene (Table 1) (Dong et al. 2005; Kim et al. 2022; Li et al. 2017; Malmgren et al. 2004; McKnight, Suzanne Hart, et al. 2008; Rajpar et al. 2002; Simmer et al. 2022). After its synthesis, the full-length DSPP protein can be immediately cleaved into two proteins, dentin sialoprotein (DSP) and dentin phosphoprotein (DPP), which are both the main components of non-collagenous proteins in the dentin matrix (MacDougall et al. 1997). DSP, primarily in the proteoglycan form, is predominantly localized in the unmineralized predentin, whereas DPP, which is phosphoserine-rich, mainly resides in the mineralized dentin (Song et al. 2008). Variants in the coding region of DSP can disrupt the transport and processing of the DSPP protein, and variants in the DPP coding region influence the process of dentin maturation (Jing et al. 2021).

In this study, we identified a novel heterozygous variant in the *DSPP* gene (NM_014208: exon2: c.38C>A: p.A13E) and characterized the dental phenotype in a Chinese family with DGI-III.

2 | Materials and Methods

2.1 | Ethical Compliance

This study was approved by the Ethics Committee of the Hospital of Stomatology, Wuhan University (2023-B15). Informed consent was obtained from the parents of the proband in accordance with the Declaration of Helsinki.

2.2 | Clinical Investigation

A 3-year-old girl with discoloration and severe wear on her teeth was referred to us at the Hospital of Stomatology, Wuhan University. We conducted oral clinical and radiological examinations for her and her family.

2.3 | Genetic Investigation

2.3.1 | Whole Exome Sequencing and Sanger Sequencing

Peripheral venous blood samples from the proband and her parents were collected to extract the genomic DNA using the Blood/Tissue/Cell Fast DNA Extraction Kit (Abclonal, Wuhan, China). The whole exome sequence (WES) analyses were performed at the Wuhan Biobank (Wuhan, China). Subsequently, the results of WES were confirmed by polymerase chain reaction (PCR) amplification (c.38C>A primers:

TABLE 1 | Summary of all reported variants in the signal peptide coding region of the *DSPP* gene and variants in other regions associated with DGI-III.

Location	cDNA (NM_014208.3)	Diagnosis	Reference
Exon2 (signal peptide region)	c.16T>G	DD-II	Rajpar et al. (2002)
Exon2 (signal peptide region)	c.38C>A	DGI-III	This article
Exon2 (signal peptide region)	c.44C>T	DGI-II	Malmgren et al. (2004)
Exon2	c.49C>T	DGI-III	Hart et al. (2007)
Exon2	c.50C>T	DGI-III	Simmer et al. (2022)
Intron2	c.52–2A>G	DGI-III	Li et al. (2017)
Exon3	c.53T>C	DGI-III	Simmer et al. (2022)
Intron3	c.135+1G>C	DGI-III	Kim et al. (2022)
Exon5	Compound variants 36bp (3599–3634) del 18bp (3715–3716) ins	DGI-III	Dong et al. (2005)

Abbreviations: Bp, base pair; Del, delete; Ins, insert.

Forward: 5′-TCCTTCATTGGCACAGGCAG-3′ and Reverse: 5′-GTGGTTTTGTTGAAACCTCACAG-3′) and Sanger sequencing to verify cosegregation with the phenotype in family members.

2.3.2 | In Silico Analysis

The online protein model prediction server AlphaFold2 (https://alphafoldserver.com/) was used to predict the conformations of proteins. SignalP 6.0 (https://services.healthtech.dtu.dk/services/SignalP-6.0/) was used to predict whether this variant altered the probability of a protein sequence serving as a signal peptide.

3 | Protein Secretion Analysis In Vitro

3.1 | Plasmid Construction

The DSP overexpression plasmid (*pcDNA3.1-EGFP- DSP*) was constructed (Gene Create). The mutant DSP_{A13E} plasmid was constructed using the Mut Express II Fast Mutagenesis Kit V2 (Vazyme). The primers were designed using CE Design (https://crm.vazyme.com/cetool/singlepoint.html) and were listed below:

Primer name	Sequence (5′-3′)
DSP_Mut A13E-F	GCAGTAGAATGGGCCATTCCAGTTCCT CAAAG
DSP_Mut A13E-R	ATGGCCCATTCTACTGCCCAAATGCAAAA ATATG

3.2 | Cell Culture and Plasmid Transfection

Human embryonic kidney 293E (HEK293E) cells were cultured in dulbecco's modified eagle medium (DMEM) (Hyclone) supplemented with 10% fetal bovine serum (FBS) (tbdScience) and 1% penicillin/streptomycin (P/S) in a 37°C incubator with 5% CO_2.

Wild-type or mutant DSP plasmids were transfected into HEK293E cells using lipo3000 (Invitrogen) according to the manufacturer's instructions.

3.3 | Western Blot (WB) Analysis and Ponceaus S Staining

After transfection for 48 h, the supernatants were collected, and the cell lysates were harvested using NP-40 (Beyotime) containing 1/100 protease inhibitor (MedChemExpress) at 4°C for 10 min. The collected samples were centrifuged, and the supernatants were mixed with 5XSDS-PAGE (Biosharp) and denatured at 95°C for 10 min. The samples were fractionated by 10% SDS-PAGE and then transferred to Trans-blot membranes (Roche). For Ponceau S staining, the membranes were incubated with Ponceau S solution (Beyotime) at room temperature for 5 min, and the images were captured. After washing three times, the membranes were blocked using 5% skimmed milk and incubated with the primary antibodies as follows: DSPP (1:2000; NOVUS) and β-actin (1:50000; ABclonal). The membranes were then incubated with the secondary antibodies, and the ECL solution (Advansta) was used for detection.

4 | Results

4.1 | Clinical Data

The primary teeth of the 3-year-old girl proband (III:1, Figure 1A) were presented with opalescent discoloration, severe attrition, and pits in tooth enamel (Figure 1B–G). Radiographically, the teeth exhibited thin dentin and abnormally enlarged pulp cavities, resembling "shell teeth" (Figure 1K). The teeth of her mother (II:2) exhibited discoloration and severe attrition, which were similar to those of the proband (Figure 2A–E). But the panoramic radiograph revealed that the teeth of her mother showed short, tapered roots with obliterated root canals (Figure 2F). Syndromic manifestations, such as osteogenesis imperfecta and hearing loss, were not observed in any family members.

Due to severe wear, the teeth of the proband showed multiple pulp exposures, which led to pulpitis or periapical periodontitis. Therefore, the proband (III:1) underwent pulp treatment, including pulpotomy, pulpectomy, and placement of stainless-steel crowns or composite resin strip crowns under general anesthesia. Intraoral and panoramic photographs after treatment were shown in Figure 1H–J,L.

4.2 | Genetic Data

Mutational analysis (see Supporting Information) showed that the proband and her mother had a c.38C>A heterozygous variant in the signal peptide coding region and a c.3240T>G heterozygous variant in the DPP coding region of the *DSPP* gene (Figure 3A). Meanwhile, her father, who was phenotypically normal, did not have these variants (Figure 3B). Previous studies have shown that a single missense variant or in-frame variant in the DPP coding region is less likely to lead to deleterious effects on the function of DSPP (Nieminen et al. 2011), so we just validated the c.38C>A variant here (Figure 3B). In addition, the genetic testing results did not show any pathogenic low-frequency variants following cosegregation in *COL1A1* and *COL1A2*, which were consistent with the proband's phenotype of no systemic disease, including osteogenesis imperfecta and hearing loss.

The c.38C>A variant has not been reported in the Genome Aggregation Database (gnomAD v4.1.0) or among the samples of East Asians' exomes. It was predicted to be D (Probably damaging) according to the Human Div database (Polyphen score = 0.999). The c.38C>A variant resulted in the substitution of the hydrophilic Glu for the hydrophobic Ala at the amino acid residue 13 located in the signal peptide domain of DSPP. To determine whether this variant influences the cleavage site of DSPP, SignalP 6.0 was used.

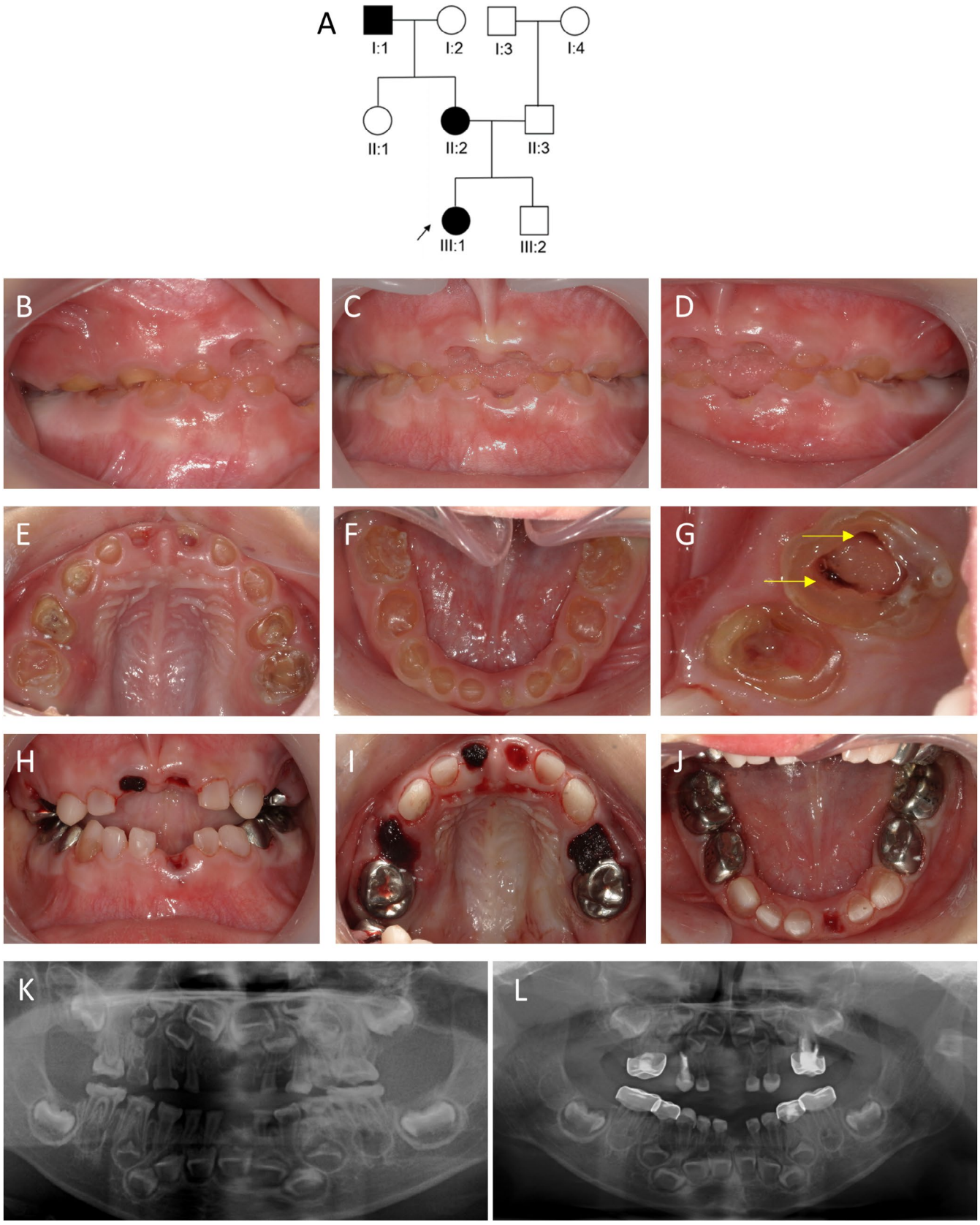

FIGURE 1 | Pedigree analysis and clinical presentation of the proband (III-1). (A) Pedigree of the family. (B–F) Intraoral photographs of the proband (III-1) before treatment. (G) The shape of the pulp chamber and the location of root canal orifices of the upper right second molar. (H–J) Intraoral photographs of the proband (III-1) immediately after treatment. (K) Panoramic photograph of the proband (III-1) before treatment. (L) Panoramic radiograph of III-1 3 months after operation.

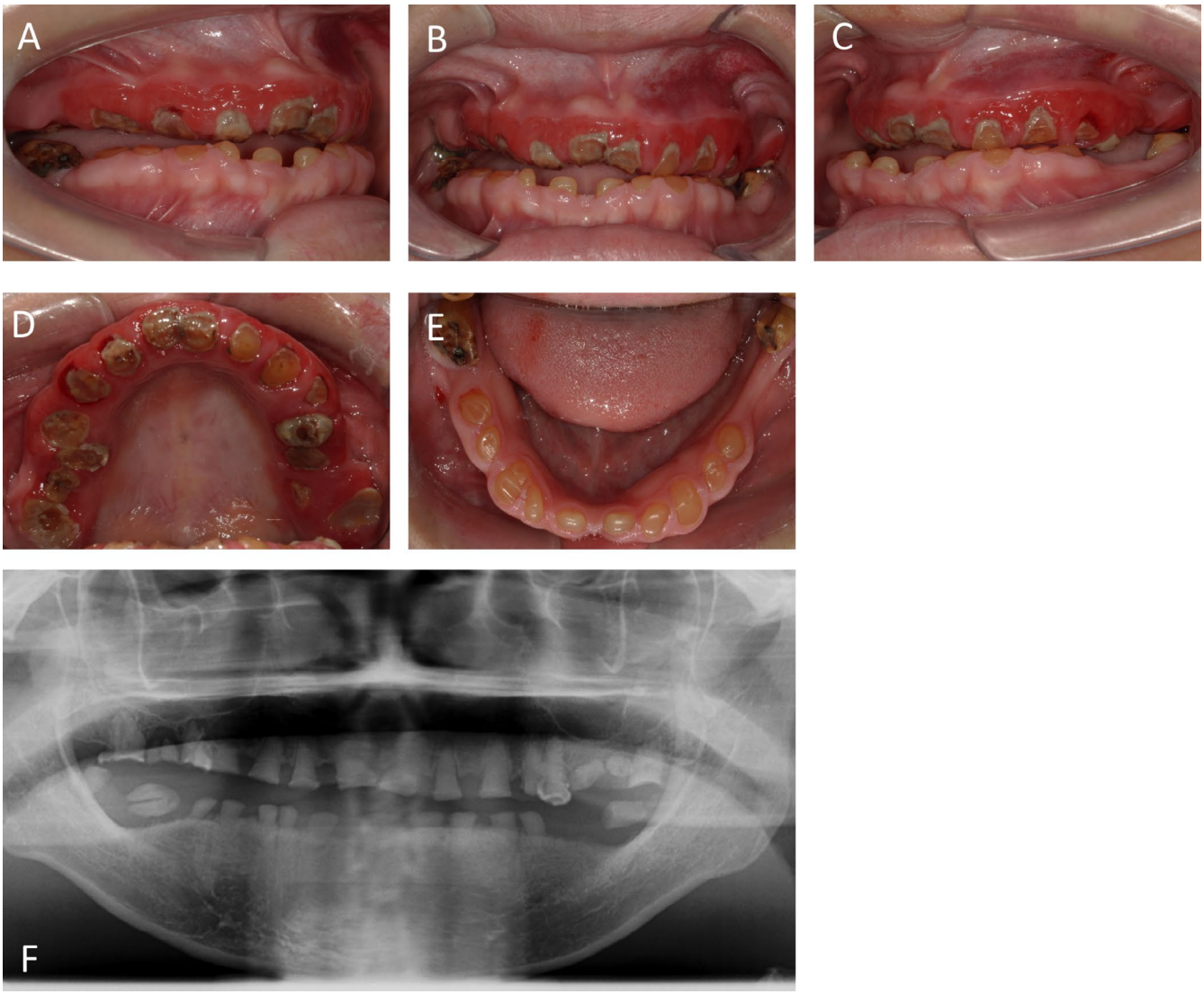

FIGURE 2 | Clinical presentation of II-2. (A–E) Intraoral photographs of II-2. (F) Panoramic photograph of II-2.

The results demonstrated that the predicted peptide cleavage site in the wild type was consistent with previous reports, while the probability of the mutated sequence functioning as a signal peptide significantly decreased (wild-type sequence: 0.9998, variant sequence: 0.4998). This suggests that the variant is likely to substantially influence the function of the signal peptide (Figure 3D). We also predicted the impact of this variation on the protein conformation by AlphaFold 2, and the results indicated that the variant did not induce apparent changes in the DSPP protein conformation by 3-D structure modeling (Figure 3C).

4.3 | Functional Analysis of the DSPP Variant

To verify whether this variant influences the secretion of DSP, we constructed the mutant DSP_{A13E} plasmid (Figure 4A) and transfected the wild-type DSP or mutant DSP_{A13E} overexpression plasmids into HEK293E cells. The results demonstrated that, different from wild-type DSP protein, mutant DSP_{A13E} was not detected in the supernatants shown by WB analysis (Figure 4B), suggesting that mutant DSP_{A13E} was not secreted extracellularly.

5 | Discussion

In this study, we identified a novel variation (NM_014208: exon2: c.38C>A: p.A13E) in the signal peptide coding domain of the *DSPP* gene in a Chinese family suffering from DGI-III. The variation was only cosegregated with affected patients in this family and was not present in the normal family members.

DSPP gene comprises 5 exons and 4 introns. Exons 2–4 encode the signal peptide domain and the N-terminal domain of DSP, while exon 5 encodes the C-terminal domain of DSP and DPP (Sun et al. 2010). The signal peptide domain of DSP spans the first 15 amino acid residues (MKIITYFCIWAVAWA). According to *The Human Gene Mutation Database* (https://www.hgmd.cf.ac.uk/ac/index.php), approximately 86 pathogenic variants in the *DSPP* gene have been identified, including nonsense, missense, and splice-site variants located in the signal peptide and DSP regions, as well as frameshift variants located in the DPP region. These pathogenic variants can be broadly categorized into two types. The first type is variants affecting the *DSPP* N′-terminus, where over 20 such variants have been reported. These variants always cluster around the signal peptide

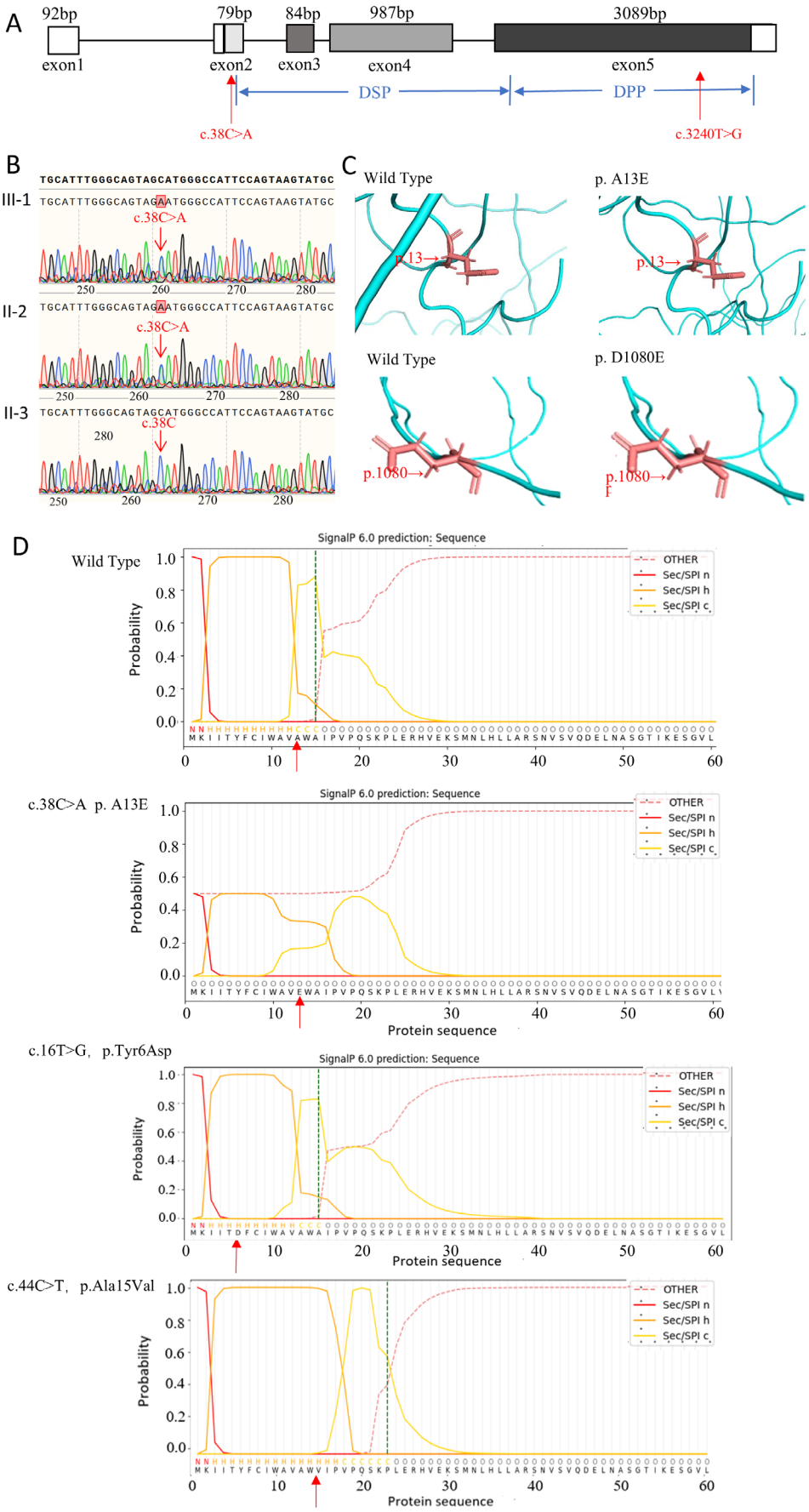

FIGURE 3 | Legend on next page.

FIGURE 3 | Sequencing chromatograms and molecular findings of the individuals in this family. (A) Schematic diagram showing the gene structure of human *DSPP* and the variation sites of the proband (red arrows). Bp, base pair. (B) DNA sequencing chromatogram of the PCR amplification product (c.38C>A present in the proband and her mother, but absent in her father). (C) 3-D structure of the DSPP protein predicted by AlphaFold2. Red arrows point to the variant sites. (D) Graphic output for the wild-type and mutant DSPP signal peptide by SignalP V6.0. c.38C>A is reported in this study, c.16T>G and c.44C>T are previously reported variants in the signal peptide region. Red arrows point to the variant sites. The green dashed line marks the signal peptide cleavage site. Genbank reference sequence of *DSPP* is NM_014208.

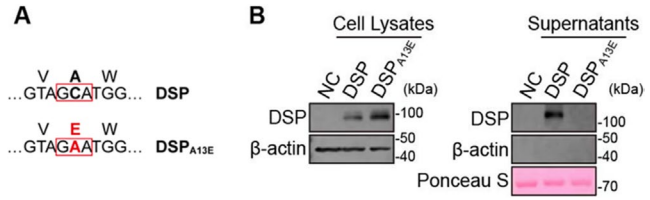

FIGURE 4 | Mutant DSP_{A13E} fails to be secreted extracellularly. (A) Plasmids encoding wild-type DSP and the mutant DSP_{A13E}. (B) WB analysis showed that wild-type DSP was seen in the supernatants of the culture medium, while mutant DSP_{A13E} was only observed in cell lysates. β-actin served as a loading control, and Ponceau S was used to indicate the total protein in the supernatants.

cleavage site (A/IPV) (Zhu et al. 2023). They affect the signal peptide cleavage, resulting in the accumulation of the mutated protein within the endoplasmic reticulum, which is deleterious to odontoblasts (Zhu et al. 2023). In this study, the results of the WB analysis showed that mutant DSP_{A13E} failed to be secreted extracellularly. Besides, a previous study has reported that the mutated DSPP protein exhibits a dominant negative effect, suggesting that it not only remains trapped in the cells but also impedes normal DSPP protein from being secreted extracellularly (von Marschall et al. 2012). This can explain the autosomal dominant inheritance of DGI.

The second type is −1 frameshift mutations in the DPP coding region, and over 30 such variants have been reported. The DPP encoding region contains more than 200 "serine–serine-asparagine (Ser-Ser-Asp)" repeats. The frameshift variants affect the hydrophilicity of this structure, leading to a significant retention of DSPP protein within the cells (McKnight, Suzanne Hart, et al. 2008). A previous study reported that the difference in phenotypes might be associated with the length of the frameshift mutation: longer mutation sequences lead to the DD phenotype, while shorter mutation sequences (< 500 codons) result in the DGI-II phenotype (McKnight, Simmer, et al. 2008). Therefore, we speculate that the single missense mutation c.3240T>G (p.D1080E) detected by WES in our report is not likely to play a role in the phenotype of the patients.

In-depth research on *DSPP* genes has improved the diagnosis and classification of hereditary dentin disorders. According to Shields' classification, DGI-III and II are isolated inherited dentin defects, and their phenotypes are different from each other. DGI-III was first observed among "Brandywine isolate" an isolated tri-racial sub-population in Southern Maryland, USA. The deciduous teeth with DGI-III often show large pulp chambers, thin dentin, and severe attrition, which leads to multiple pulp exposures. However, for the permanent teeth, the pulpal chambers are either smaller than normal or even completely obliterated (Witkop 1988). According to the modified variants-based classification by Dr. Simmer, patients who possess a dominant 5′-*DSPP* variant but except those in the signal peptide coding region, are at a high risk of experiencing rapid enamel attrition, an increased risk of pulp exposure, and dental abscess. These patients should be diagnosed with DGI-III (Simmer et al. 2022). The reason for excluding signal peptide segment mutations may be due to the limited cases and the differences between their phenotypes. Till now, only two families with variations in the signal peptide region of the *DSPP* gene have been reported, with the phenotypes of DGI-II (c.44A>T, p. Ala15Val) and DD-II (c.16T>G, p. Tyr6Asp), respectively (Malmgren et al. 2004; Rajpar et al. 2002). In our study, c.38C>A (p.A13E) is located in the signal peptide domain. Based on the above analysis and the clinical manifestations of the patients, we diagnose the family in our report with DGI-III. According to the prediction from SignalP 6.0, the c.38C>A variant displayed the most significant impact on the cleavage site of the DSPP signal peptide, which may explain why the phenotype of the family in this study is more severe compared to the other known families with signal peptide variants (Figure 3D).

However, some researchers believe that the enlarged pulp chambers and root canals in the deciduous dentition of DGI-III are a variant phenotype of DGI-II since the phenotype of the permanent teeth with DGI-III are the same as DGI-II. DGI-III is therefore considered a special form of DGI-II, and DGI-II should be used to describe both DGI type II and type III (Kim et al. 2005; Song et al. 2006). Teeth with DGI-III exhibit more severe attrition than those with DGI-II, which can cause increased external stimulation to the pulp of DGI-III permanent teeth with age, leading to the subsequent excessive reparative dentin formation. We therefore believe that the manifestation of root canal obliteration in DGI-III permanent dentition may not be directly associated with gene variant spots. For DGI-II young permanent teeth, their pulp chambers and root canals start to become narrower before eruption (de La Dure-Molla et al. 2015). Till now, no report describes the phenotype of DGI-III young permanent teeth before the eruption. Investigations on the phenotype of the developmental permanent teeth with DGI-III are needed.

Clinically, dentin developmental defects in DGI-III patients are irreversible, and there still lack comprehensive management strategies, which leads to a poor clinical prognosis. Timely intervention and comprehensive treatment, including pulp treatment and stainless-steel crowns (coating the tooth surface to reduce wear), are necessary. Unfortunately, these treatments do not always achieve the desired effects, so gene therapy has been used to prevent dentin developmental defects in mice, which may be a potential strategy for treating hereditary dentin disorders in humans in the future (Fu et al. 2023).

6 | Conclusion

DGI-III is a rare type of hereditary dentin defect characterized by enlarged pulp chambers and thin dentin. Here, we report a family with DGI-III caused by a novel variant of the *DSPP* gene, which broadens the phenotypes associated with variants in the signal peptide region of *DSPP*. Further investigations are needed to unveil the mechanisms by which variants in the *DSPP* signal peptide coding region lead to different phenotypes.

Author Contributions

Yan Wang collected clinical data, performed the experiments, and wrote the original draft. Yuzhe Ding and Ximin Xu performed the experiments and analyzed the data. Guohua Yuan enrolled the family, analyzed the data, supervised the project, and edited the manuscript.

Acknowledgments

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Ethics Statement

This study was approved by the Ethics Committee of Stomatological Hospital Wuhan University (2023-B15).

Consent

The experiments were conducted with the understanding and written consent of each participant. All participants have provided their informed consent for their images to be used in this publication.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.