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ORIGINAL ARTICLE

Phenotype and genotype analyses in seven families with dentinogenesis imperfecta or dentin dysplasia

F Li[1], Y Liu[1], H Liu[1], J Yang[1], F Zhang[2], H Feng[1]

[1]*Department of Prosthodontics, National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing Key Laboratory of Digital Stomatology, Peking University School and Hospital of Stomatology, Beijing;* [2]*Department of Pediatrics, Peking University School and Hospital of Stomatology, Beijing, China*

OBJECTIVE: Hereditary dentin defects can be categorised into two classes according to their clinical manifestations: dentinogenesis imperfecta (DGI), which includes three types (DGI-I, DGI-II and DGI-III), and dentin dysplasia (DD), which includes two types (DD-I and DD-II). This study investigated the phenotypic characteristics and genetic causes of hereditary dentin defects in seven Chinese families.

MATERIALS AND METHODS: Seven families affected with DGI-II, DGI-III or DD-II were enrolled. Clinical examinations were performed to determine the phenotypic characteristics, and DNA samples were collected for Sanger sequencing.

RESULTS: Clinical diagnoses revealed DGI-II in five families, DGI-III in one family and DD-II in one family. Variants of the *dentin sialophosphoprotein* (*DSPP*) gene were found in six of the seven families. Of these, c.52G>T was identified in two families. Each of the remaining four families had a different variant: c.2684delG, c.52-2A>G, c.1874-1877delACAG and c.3509-3521del13bp; the last three variants were novel.

CONCLUSIONS: This is the first study to analyse all three important types of hereditary dentin defect and include comprehensive genetic analyses of both dentin sialoprotein and dentin phosphoprotein in Chinese families. This study expands the spectrum of *DSPP* variants, highlighting their associated phenotypic continuum.

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Keywords: dentin dysplasia; dentinogenesis imperfecta; dentin sialophosphoprotein; variants

Introduction

Hereditary dentin defects show autosomal-dominant transmission patterns that mainly affect dentin. According to the classification system proposed in 1973 (Shields *et al*, 1973), hereditary dentin defects are classified into two categories based on clinical and radiographic features: dentinogenesis imperfecta (DGI) and dentin dysplasia (DD) (Shields *et al*, 1973).

Dentinogenesis imperfecta-I describes a phenotype in which osteogenesis imperfecta (OI) occurs concurrently with dentin defects, with the dentin phenotype in cases with DGI-I appearing similar to that found in cases with DGI-II (Shields *et al*, 1973). DGI-I is currently considered a syndromic phenotype. DGI-II is the most prevalent type of inherited dentin defect. Its features include opalescent discolouration, bulbous crowns, and obliterated pulp chambers and root canals in both deciduous and permanent dentitions. The enamel chips easily from the underlying dentin due to an abnormal enamel–dentin junction, and this causes attrition of dentin to varying degrees (MacDougall *et al*, 2006). DGI-III is characterised by multiple pulp-exposed and shell-like teeth. Although originally identified in Brandywine triracial isolates, cases in other races have since been reported (Hart and Hart, 2007; Kim and Simmer, 2007). DD-I has a rather low prevalence with unique features; the teeth show normal colour and shape but have extremely short roots and obliterated pulp chambers. The deciduous teeth in DD-II and DGI-II cases display the same phenotype, whereas the permanent teeth in DD-II cases have normal colour and shape but display thistle tube-shaped or obliterated pulp chambers and pulp stones on radiographic analysis (Shields *et al*, 1973).

Several other genes associated with dentin formation are considered candidate genes for hereditary dentin defects; however, only mutations in *DSPP* have been shown to result in DGI-II, DGI-III and DD-II (Xiao *et al*, 2001; Zhang *et al*, 2001, 2007, 2011; Rajpar *et al*, 2002; Kim *et al*, 2004, 2005; Malmgren *et al*, 2004; Dong *et al*, 2005; Holappa *et al*, 2006; Song *et al*, 2006, 2008; Lee *et al*, 2008, 2009, 2011a, 2013; McKnight *et al*, 2008a,b; Kida *et al*, 2009; Wang *et al*, 2009; Bai *et al*, 2010;

Correspondence: Hailan Feng, Department of Prosthodontics, Peking University School and Hospital of Stomatology, 22 Zhongguancun Nandajie, Haidian District, Beijing 100081, China. Tel: +86 10 82195232, Fax: +86 10 62173402, E-mail: kqfenghl@bjmu.edu.cn.
The GenBank accession numbers: c.52-2A>G: KX858802, c.1874-1877delACAG: KX858803, c.3509-3521del13bp: KX858804.
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Nieminen *et al*, 2011; Li *et al*, 2012; Liu *et al*, 2016; Yang *et al*, 2016). Therefore, the three diseases seem to be allelic with varying phenotypes. DD-II is considered a mild hereditary dentin defect, whereas DGI-III is a severe form.

In this study, we collected seven Chinese families with phenotypes of hereditary dentin defects. By thorough sequence analysis, *DSPP* variants were identified in six families. This study presents all three important types of hereditary dentin defects (DGI-II, DGI-III and DD-II) and provides a comprehensive genetic analysis of both the DSP and DPP regions of DSPP.

Materials and methods

Subjects

The study protocol was approved by the Ethics Committee of Peking University Health Science Centre (PKUSSIRB-2013011). Informed consent was obtained from each subject or his/her guardian in keeping with the Declaration of Helsinki. This study enrolled seven Chinese families with hereditary dentin defects. All available family members were examined clinically. To confirm the diagnosis, radiography was performed in subjects suspected to be affected.

DNA sequencing

Genomic DNA of each subject was isolated from peripheral blood lymphocytes using a BioTek DNA Whole-blood Mini Kit (BioTek, Beijing, China). The entire coding region and intron–exon junctions of the DSPP gene were amplified by polymerase chain reaction (PCR) with *Taq* PCR Master Mix (BioTek), as described previously (Song *et al*, 2008). The products were sent to Tsingke Biological (Beijing, China) and sequenced as described previously (Li *et al*, 2015). When no pathogenic variants were seen in the DSP region, we performed TOPO®-TA cloning of the DPP region. The purified PCR products were reacted with pClone007 Vector (Tsingke) and then cloned into competent cells. After reacting on ice, in a 42°C water bath, and on ice again, the reaction was incubated in LB growth medium. The cells were spread on a plate and grown at 37°C overnight. Selected colonies were inoculated in LB/Amp growth medium and shaken at room temperature overnight. The plasmids were digested with restriction enzymes to analyse the mutations and then sequenced using primers M13F (TGTAAAACGACGGCCAGT) and M13R (CAG GAAACAGCTATGACC) (McKnight *et al*, 2008a).

Sequence analyses were performed using SeqMan Pro genetic analysis software (DNASTAR, Madison, WI, USA). The nucleotides were numbered starting from the A of ATG in the human DSPP reference sequence (NM_014208).

Results

Clinical findings

Of the seven Chinese families enrolled in our study, five showed an autosomal-dominant transmission pattern, while the remaining two patterns (E and F) were sporadic.

In family A, amber yellow teeth and moderate attrition were noticeable (Figure 1a) in the proband. The typical features of bullous crowns and obliterated pulp cavities were also evident (Figure 1b).

In family B, nearly all of the teeth of the proband had been treated with crowns, and the periapical radiographs showed obliterated root canals (Figure S1a, c). However, her 4-year-old daughter's teeth showed amber opalescent discolouration. The teeth were severely worn, and some were missing due to wear. A periapical radiograph showed obliterated root canals and pulp chambers (Figure S1b, d).

Families C (Figure S1e, f), D (Figure S1g, h) and E showed classical and typical manifestations of DGI-II, with features that included opalescent grey or yellow discolouration and attrition to varying degrees. All available affected members showed constriction of the neck of the teeth and pulpal obliteration on radiographs.

In family F (Figure 2), the 4-year-old girl proband had primary teeth that showed yellow discolouration and severe attrition (Figure 2a), similar to the proband's daughter in family B. However, a panoramic radiograph revealed abnormally enlarged pulp cavities (Figure 2b, c), which differed from the five families described above.

In family G (Figure 3), a 9-year-old boy had amber yellow discolouration and attrition of the cusps of his primary canines, but normal permanent teeth in both colour and shape (Figure 3a, b). However, the periapical radiographs revealed obliterated pulp cavities in all teeth (Figure 3c–e).

None of the affected individuals in the above families had symptoms of hearing loss, bone defects or other relevant diseases. Furthermore, none had been diagnosed with OI or other syndromes that included the dentin defect phenotype. Table S1 summarises the clinical features of the available affected individuals.

According to the clinical manifestations, DGI-II was determined in families A, B, C, D and E; DGI-III was identified in family F; and DD-II was diagnosed in family G.

Mutation results

In the DSP coding region, we found two different missense variants in three families, and in the DPP coding region, we identified three frameshift variants in three families (Table 1). Three of the variants were novel and were not found in databases such as the Exome Sequencing Project, 1000 Genomes Project and Exome Aggregation

Table 1 Pathogenic variants identified in this study

Family	*Phenotype*	*Location*	*cDNA*	*Type*	*Zygosity*	*Protein*	*Previous reports*	*ACMG classification*
A	DGI-II	Exon 3	c.52G>T	Missense	Heterozygous	p.V18F + p.V18_Q45del	Xiao *et al* (2001),	–
B	DGI-II	Exon 3	c.52G>T	Missense	Heterozygous	p.V18F + p.V18_Q45del	Kim *et al* (2005), Song *et al* (2006), Holappa *et al* (2006)	–
C	DGI-II	Exon 5	c.2684delG	Frameshift	Heterozygous	p.S895MfsX418	Song *et al*, 2008	–
D	DGI-II	Exon 5	c.3509-3521del13bp	Frameshift	Heterozygous	p.D1170AfsX139	Novel	Pathogenic
F	DGI-III	Intron 2	c.52-2A>G	Splice site	Heterozygous	p.V18_Q45del	Novel	Pathogenic
G	DD-II	Exon 5	c.1874-1877delACAG	Frameshift	Heterozygous	p.D625AfsX687	Novel	Pathogenic

ACMG, American College of Medical Genetics.

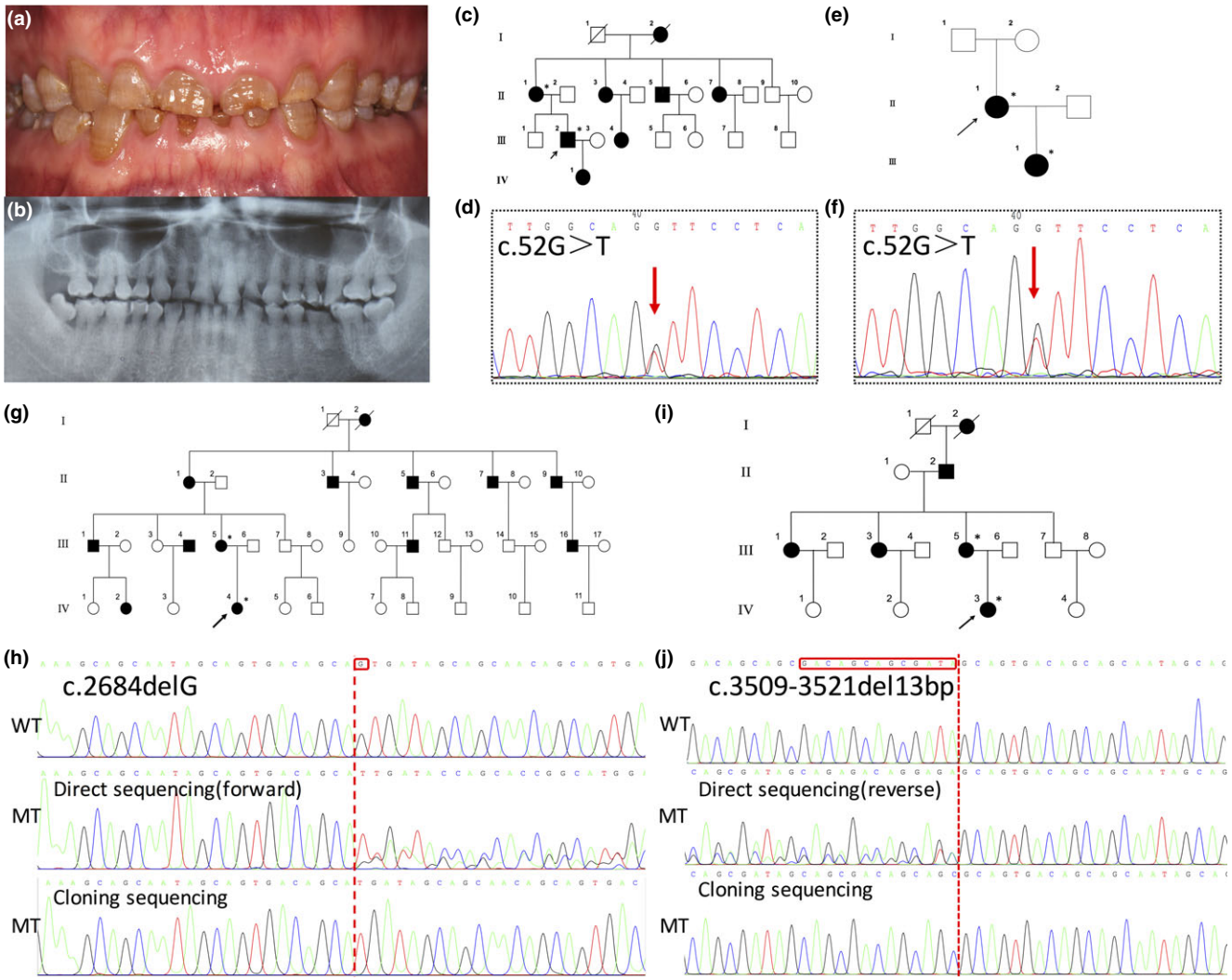

Figure 1 Clinical presentations, radiographic findings, family pedigrees and variant analyses in families A, B, C and D with DGI-II. (**a**) Photograph of the proband III-2 in family A shows opalescent yellow discolouration and attrition of teeth. (**b**) Panoramic radiograph of III-2 in family A shows obliterated pulp chambers and root canals. (**c**) Pedigree of family A. (**d**) Sanger sequencing result of family A shows a double peak indicating c.52G>T. (**e**) Pedigree of family B. (**f**) Sanger sequencing result of family B shows a double peak indicating c.52G>T. (**g**) Pedigree of family C. (**h**) TOPO®-TA cloning sequencing shows a 1-bp deletion in the variant. (**i**) Pedigree of family D. (**j**) TOPO®-TA cloning sequencing shows a 13-bp deletion in the variant [Colour figure can be viewed at wileyonlinelibrary.com]

Consortium. In addition, they were predicted to be pathogenic according to the American College of Medical Genetics guidelines (Richards *et al*, 2015; Table S2).

Families A (Figure 1d) and B (Figure 1f) contained a variant that was identified in the first codon of exon 3, c.52G>T. Variations at this position have been reported (Xiao *et al*, 2001; Kim *et al*, 2005; Holappa *et al*, 2006; Song *et al*, 2006), and *in vitro* splicing assays have confirmed that this variant results in deletion of exon 3 (Lee *et al*, 2011b).

In family C, a frameshift variant in exon 5 was caused by a 1-bp deletion, c.2684delG (Figure 1h). This result was also found in a Chinese family in 2008 (Song *et al*, 2008), causing amino acid changes beginning at position 895 from Ser-Ser-Asp repeats to Ala-Ala-Ile repeats.

Family D displayed a novel frameshift variant in exon 5, c.3509-3521del13bp (Figure 1j). This variant caused a 13-bp deletion near the C-terminus, and the amino acids beginning at position 1170 changed from Ser-Ser-Asp repeats to Ala-Ala-Ile repeats.

Family E displayed no pathogenic variants, but we found several polymorphisms in *DSPP* (Table S3).

Family F contained a novel variant in intron 2, c.52-2A>G (Figure 2e), which occurred at a splicing acceptor site. We speculate that this variant may result in removal of part or all of exon 3.

Family G exhibited a different novel frameshift variant in exon 5, c.1874-1877delACAG (Figure 3g). This variant contained a 4-bp deletion near the N-terminus of DPP, causing downstream changes to amino acids rich in alanine, valine and isoleucine, in place of aspartic acid and serine.

Discussion

Hereditary dental diseases include disorders affecting formation of the enamel and dentin and anomalies in the

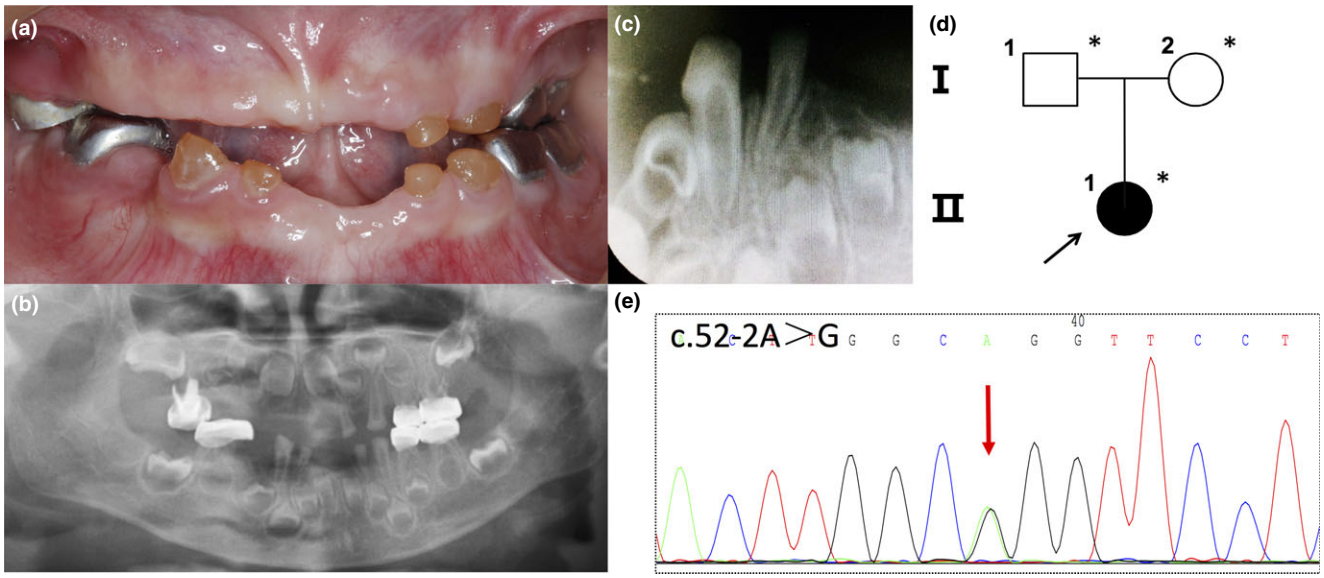

Figure 2 Clinical presentation, radiographic findings, family pedigree and variant analysis in family F with dentinogenesis imperfecta (DGI)-III. (**a**) Photograph of the proband, II-1, shows amber yellow discolouration and severe attrition of the teeth. (**b, c**) Panoramic and periapical radiographs of II-1 show enlarged pulp chambers and root canals. (**d**) Family pedigree. (**e**) Sanger sequencing result shows a double peak indicating c.52-2A>G [Colour figure can be viewed at wileyonlinelibrary.com]

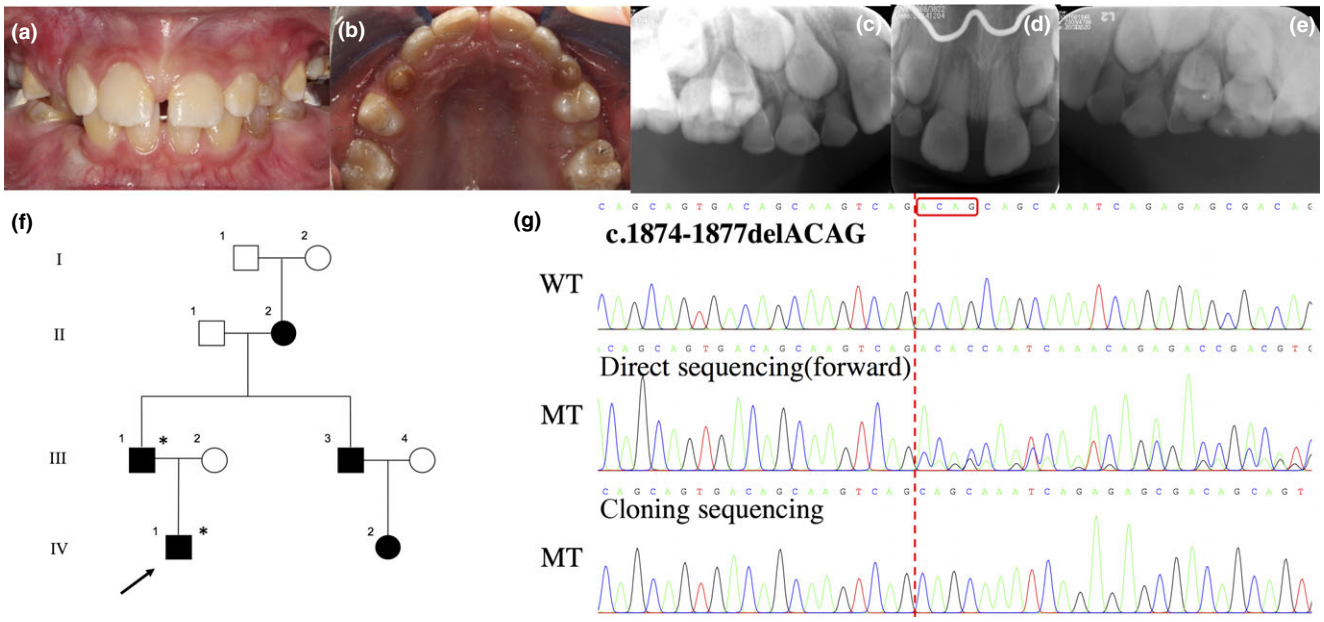

Figure 3 Clinical presentation, radiographic findings, family pedigree and variant analysis in family G with dentin dysplasia (DD)-II. (**a, b**) Photograph of the proband, IV-1, shows opalescent yellow discolouration and attrition in the primary canines and normal colour and shape in the permanent teeth. (**c–e**) Periapical radiographs of IV-1 show obliterated pulp chambers and root canals in both primary and permanent teeth. (**f**) Family pedigree. (**g**) TOPO®-TA cloning sequencing shows a 4-bp deletion in the variant. Black arrows: probands; asterisks: the individuals collected in this study; red arrows: positions of the variant; red boxes: deleted nucleotides; WT, wild type; MT, mutant type [Colour figure can be viewed at wileyonlinelibrary.com]

numbers of teeth. The three main corresponding diseases are amelogenesis imperfecta, dentin defects and tooth agenesis. Non-syndromic hereditary dentin defects affect mainly the structure of dentin, an essential component of teeth. The prevalence of DGI is between 1:6000 and 1:8000; DD-I is 1:100 000 (Witkop, 1957) that of amelogenesis imperfecta ranges from 0.025% to 0.43% (Sedano, 1975; Sundell and Koch, 1985; Backman and Holm, 1986; Altug-Atac and Erdem, 2007; Gupta *et al*, 2011; Shokri *et al*, 2014); and oligodontia, a severe form of tooth agenesis, occurs in approximately 0.25% of the Chinese population (Feng, 2011) and 0.14–0.30% of Caucasians (Dhanrajani, 2002). Therefore, hereditary dentin defects are relatively rare compared with other dental diseases and it is difficult to collect large numbers of cases. Most previous clinical reports describing hereditary dentin

defects included one or two families, making it difficult to study and compare the diseases. Only four reports included more than five families (Holappa *et al*, 2006; McKnight *et al*, 2008b; Song *et al*, 2008; Nieminen *et al*, 2011). The current study included seven families and contained all three important types of defects (DGI-II, DGI-III and DD-II), which was essential for analysing and comparing the phenotypic and genetic characteristics.

Mutations in the *DSPP* gene have been confirmed to result in non-syndromic hereditary dentin defects, including DGI-II, DGI–III and DD-II (Xiao *et al*, 2001; Zhang *et al*, 2001). Currently, 40 *DSPP* mutations have been detected in people of different ethnicities (Table S4). Of these, 17 mutations were in the DSP region, with most being missense or nonsense mutations, while 23 variants were frameshift mutations located in the DPP region. Our study investigated the clinical manifestations of inherited dentin defects and identified variants in the *DSPP* gene. We found five different variants in six families, and three of these variants were novel.

Families A and B were determined as DGI-II, and both families showed obliteration of pulp cavities. The teeth in the affected individuals in family A showed attrition on radiographs, while those in family B had been treated with crowns. Variant c.52G>T was found in these two families, which has also been identified in several other studies (Xiao *et al*, 2001; Kim *et al*, 2005; Holappa *et al*, 2006; Song *et al*, 2006). These results suggest that this position is a mutational 'hot spot' in the *DSPP* gene. Most of the affected individuals demonstrated the DGI-II phenotype; however, one was diagnosed as DGI-III, indicating that the same mutation can cause different phenotypes. *In vitro* splicing assays have confirmed that this mutation creates aberrant pre-mRNA splicing events resulting in deletion of exon 3 (Lee *et al*, 2011b). Although it may not cause significant frameshift or premature effects on the downstream sequence, it could influence the signal peptide cleavage site by changing the amino acid residues, disturbing cleavage, which could affect normal DSPP function by interrupting folding and secretion (Wang *et al*, 2011).

The proband in family F was a 4-year-old girl, and the oral manifestation of discolouration and attrition appeared similar to the proband's daughter in family B. However, radiographic analysis revealed enlarged pulp chambers and root canals in the former, while the latter presented obliterated pulp cavities. Therefore, the proband in this family was diagnosed as DGI-III and the proband's daughter in family B was diagnosed as DGI-II. However, when the permanent dentition of the proband erupts, she may have a different diagnosis. If the phenotype is the same as that of her primary dentition, the diagnosis is still DGI-III. If the colour of teeth is similar to that of her primary teeth but the radiograph shows obliterated pulps, she would be diagnosed with DGI-II. If her permanent teeth are of normal shape and colour but have obliterated pulps, this would be consistent with a diagnosis of DD-II. Neither parent of the proband showed signs of dentin defects (data not shown), and they had no variants at c.52-2A>G. Therefore, the inheritance mode in family F was sporadic, and the variant identified in this family was novel. As the variant occurred at the splicing acceptor site, we speculate that it may affect pre-mRNA splicing, perhaps causing partial or complete removal of exon 3, similar to the mutational effect of c.52G>T.

Successful sequencing of the highly repeated DPP was an important step in the genetic study of hereditary dentin defects (McKnight *et al*, 2008a,b; Song *et al*, 2008; Lee *et al*, 2011a; Nieminen *et al*, 2011; Yang *et al*, 2016). Because the numbers of tandem Ser-Ser-Asp repeats differs among normal human individuals, indels of 9 bp, 18 bp or similar variants rarely cause pathological results. We identified three frameshift variants in our study: c.2684delG in family C, c.3509-3521del13bp in family D and c.1874-1877delACAG in family G. The 1-, 4- and 13-bp deletions corresponded to the effect of a 1-bp loss. All of these frameshift mutations encoded downstream amino acids that were rich in hydrophobic alanine, valine and isoleucine amino acids instead of hydrophilic aspartic acid and serine (McKnight *et al*, 2008b). The −1 frameshift mutations may cause retention of the mutant protein in the cell, presumably in the rough endoplasmic reticulum, which may promote protein linkage and entrapment of wild-type DSPP (von Marschall *et al*, 2012).

Previous studies showed that frameshift mutations in the anterior region of DPP correlated with the DD-II clinical phenotype, a less severe form of hereditary dentin defect, while mutations in the posterior region were associated with the more severe form, DGI-II (McKnight *et al*, 2008b; Song *et al*, 2008). Our data support this correlation. The primary teeth of the proband in family G showed discolouration and attrition, while the permanent teeth seemed normal in colour and shape. The variant in this family was c.1874-1877delACAG, and the phenotype was consistent with the DD-II diagnosis. Families C and D showed classical manifestation of DGI-II, and the variants identified were c.2684delG and c.3509-3521del13bp, respectively. This location is closer to the C-terminus of DPP compared with c.1874-1877delACAG. The exact reason for this correlation between the frameshift mutation location and the clinical features remains unknown. Nieminen *et al* (2011) speculated that a frameshift mutation located further downstream results in a more severe phenotype due to the longer hydrophilic DPP sequence with a shorter hydrophobic tail. Such variation may cause greater disruption in normal DPP function or confer a higher tendency to be misguided during transport.

Family E showed no pathogenic variants; however, several variants have been confirmed as polymorphisms in *DSPP*. Song *et al* (2008) reported eight families with exclusion mutations in the DSP region; they found mutations in the DPP region in five families, while the remaining three families showed no pathogenic mutations. Wang *et al* (2012) found a mutation in *COL1A2* in a DGI family without bone defects, and they suggested that *COL1A1* and *COL1A2* should be considered candidate genes in isolated DGI cases without a *DSPP* mutation. However, we found several polymorphisms, but no pathogenic mutations, in these two genes in Family E. Plausible explanations include other genes responsible for these dentin defects (whole-exon sequencing could be used to investigate new underlying genes); polymorphisms may mitigate a defect when they appear together (this possibility could

be investigated using *in vitro* assays and/or animal experiments); and copy number variation may lead to the occurrence and development of disease to some degree (Almal and Padh, 2012). However, no previous studies have described such variations in hereditary dentin defects; these possibilities require further study.

This study identified *DSPP* variants in six of seven Chinese families with hereditary dentin defects, of which three variants were novel. This is the first study to present all three important types of hereditary dentin defects (DGI-II, DGI-III and DD-II) and perform comprehensive genetic analyses of both DSP and DPP regions of DSPP in Chinese families. This study expanded the spectrum of *DSPP* variants and highlighted the phenotypic continuum associated with *DSPP* variants. Further research is required to investigate the cellular and molecular mechanisms of these variants.

Conflicts of interest

None to declare.

Acknowledgements

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Author contributions

Fang Li, Yang Liu, Haochen Liu, Hailan Feng, Jingwen Yang and Fangfei Zhang collected and analysed the patient information. Hailan Feng and Fang Li designed the study. Fang Li performed the experiments. Fang Li, Yang Liu, Haochen Liu and Hailan Feng prepared the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Clinical presentations and radiographical findings in families B, C, and D with DGI-II.

Table S1 Clinical features of the affected individuals in this study.

Table S2 Classification of the novel variants according to ACMG guideline.

Table S3 Polymorphisms found in *DSPP* in the proband of family E.

Table S4 Summary of the mutations in *DSPP*.