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Novel Frameshift Mutations in *DSPP* Cause Dentin Dysplasia Type II

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The current classification system of hereditary dentin defects was proposed in 1973 (Shields et al., 1973) based on clinical and radiographic information without knowledge of the underlying molecular pathophysiology: three types of dentinogenesis imperfecta (DGI-I, DGI-II, and DGI-III) and 2 types of dentin dysplasia (DD-I and DD-II).

Dentin sialophosphoprotein (DSPP) is the most abundant non-collagenous component in dentin and a member of the acidic secretory calcium-binding phosphoprotein (SCPP) gene family (Kawasaki & Weiss, 2006). After DSPP is synthesized, it undergoes extensive posttranslational modifications (Yamakoshi et al., 2011) and is cleaved into two major functional units: an N-terminal fragment called dentin sialoprotein (DSP) and a C-terminal fragment known as dentin phosphoprotein (DPP) (Yamakoshi & Simmer, 2018). Mutations in *DSPP* have been identified to cause DGI-II, DGI-III and DD-II (Kim & Simmer, 2007), and there are some overlapping phenotypes in some cases (McKnight et al., 2008a). Therefore, it has been suggested that these three diseases are not separate entities but a spectrum of the disease depending on the degree of severity (Beattie et al., 2006).

A genotype-phenotype correlation with regard to the frameshift mutations in the DPP region was suggested when N-terminal frameshifts in the DPP region were observed in

association with DD-II, and more C-terminal frameshift mutations were found to cause DGI-II (McKnight et al., 2008b). DD-II like frameshift mutations would generate shorter negative charged repeats than DGI-II like mutations in the N-terminus of the DPP. A reduction of the interaction of the mutant DSPP in the ER with the wild type DSPP through the Ca^{2+} bridge in the DD-II like mutation, enabling the secretion of the wild type DSPP into the dentin matrix, was suggested as a molecular basis of the genotype-phenotype correlation (von Marschall et al., 2012).

In this study, we recruited four families with DD-II and performed a mutational analysis, including Sanger sequencing of exons and exon-intron boundaries of *DSPP* and cloning of *DPP* repetitive sequence. The novel mutation identified in family 1 and 2 (Turkish families) [c.2134delA, p.(Ser712Alafs*602)] confirms the previous genotype-phenotype correlation and extends the range about 70 bp down to the C-terminus (Figure 1). The previous exception to the correlation was a DD-II family caused by the c.3135delC [p.(Ser1045Argfs*269)] mutation (Yang et al., 2016), and there were some features of DGI-II such as slight discoloration, bulbous crowns and obliterated pulp chambers in some teeth. Interestingly, family 3 (Korean family) has an overlapping phenotype with a slight discoloration in the lingual side of the anterior teeth in the proband on the DD-II phenotype, and the location of the mutation [c.3480_3481insCTGCT, p.(Asp1161Leufs*155)] is similar to a previous family. Family 4 (Korean family) has an extremely mild, characteristic DD-II phenotype, and the mutation [c.3179delG, p.(Ser1060Thrfs*254)] is also close to the above 2 families. This study confirms the previous genotype-phenotype correlation and extends the range of the DD-II associated N-terminus of the DPP region; however, it also provides additional exceptions to the previously DGI-II associated C-terminus of the DPP region.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

J.W. Lee, J. Hong, contributed to analysis and interpretation, drafted manuscript, critically revised manuscript; F. Seymen, M. Koruyucu, N. Tuloglu, S. Bayrak, J.-C. Lee, contributed to conception and design, and data acquisition, critically revised manuscript; Y.J. Kim, J. Kang, contributed to data analysis and interpretation, critically revised manuscript; J.-S. Song, T.J. Shin, H.-K. Hyun, Y.-J. Kim, J.-C. Park, contributed to the conception, critically revised the manuscript; J. Hu, J. Simmer, J.-W. Kim, contributed to the conception, design, data acquisition, analysis, and interpretation, drafted manuscript, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

REFERENCES

Beattie ML, Kim JW, Gong SG, Murdoch-Kinch CA, Simmer JP and Hu JC (2006). Phenotypic variation in dentinogenesis imperfecta/dentin dysplasia linked to 4q21. *J Dent Res* **85**: 329-33.

Kawasaki K and Weiss KM (2006). Evolutionary genetics of vertebrate tissue mineralization: the origin and evolution of the secretory calcium-binding phosphoprotein family. *J Exp Zool B Mol Dev Evol* **306**: 295-316.

Kim JW and Simmer JP (2007). Hereditary dentin defects. *J Dent Res* **86**: 392-9.

McKnight DA, Simmer JP, Hart PS, Hart TC and Fisher LW (2008a). Overlapping DSPP mutations cause dentin dysplasia and dentinogenesis imperfecta. *J Dent Res* **87**: 1108-11.

McKnight DA, Suzanne Hart P, Hart TC, Hartsfield JK, Wilson A, Wright JT and Fisher LW (2008b). A comprehensive analysis of normal variation and disease-causing mutations in the human DSPP gene. *Hum Mutat* **29**: 1392-404.

Shields ED, Bixler D and el-Kafrawy AM (1973). A proposed classification for heritable human dentine defects with a description of a new entity. *Arch Oral Biol* **18**: 543-53.

von Marschall Z, Mok S, Phillips MD, McKnight DA and Fisher LW (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. *J Bone Miner Res* **27**: 1309-21.

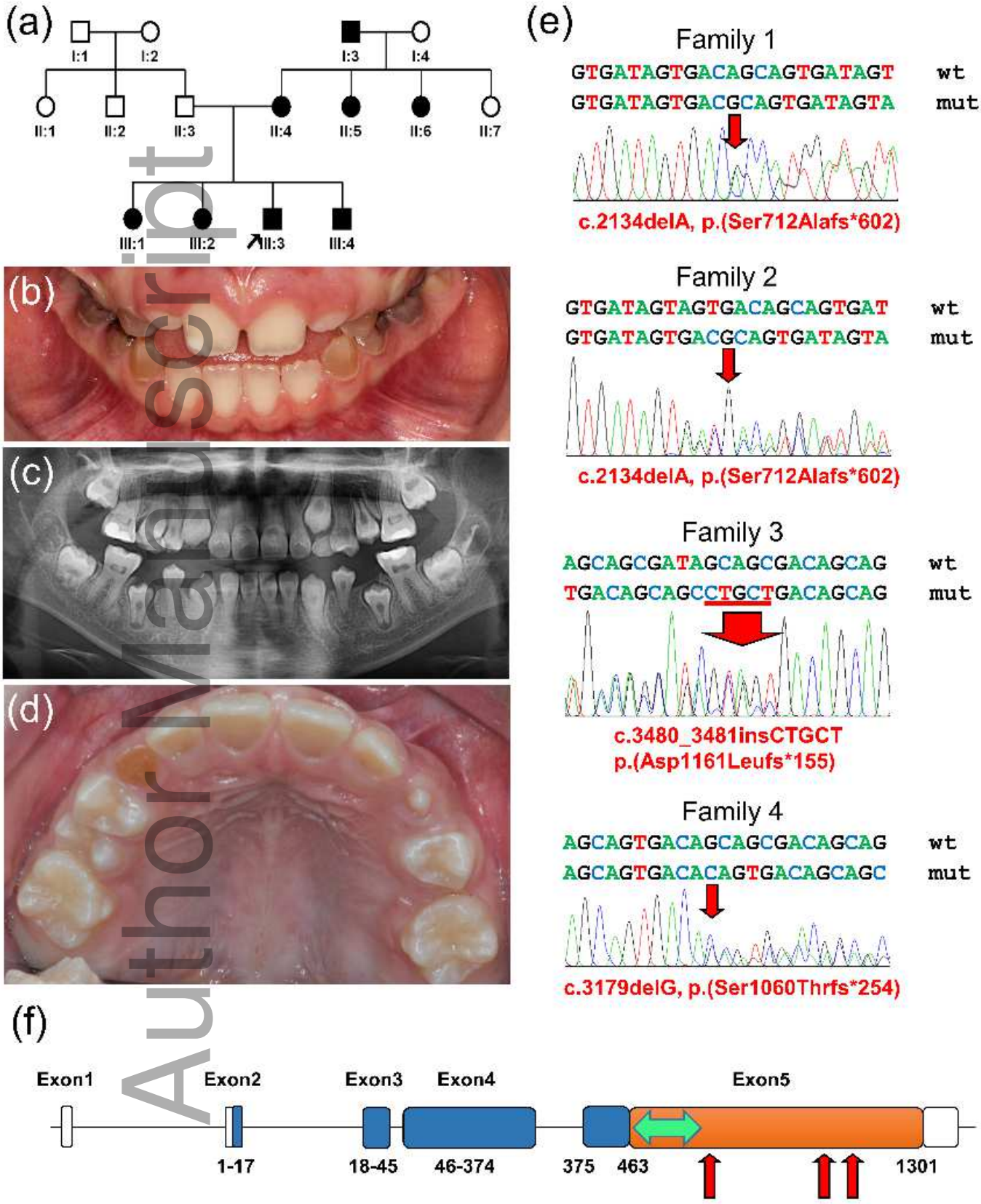
Yamakoshi Y, Nagano T, Hu JC, Yamakoshi F and Simmer JP (2011). Porcine dentin sialoprotein glycosylation and glycosaminoglycan attachments. *BMC Biochem* **12**: 6.

Yamakoshi Y and Simmer JP (2018). Structural features, processing mechanism and gene splice variants of dentin sialophosphoprotein. *The Japanese dental science review* **54**: 183-196.

Yang J, Kawasaki K, Lee M, Reid BM, Nunez SM, Choi M, Seymen F, Koruyucu M, Kasimoglu Y, Estrella-Yuson N, Lin BP, Simmer JP and Hu JC (2016). The dentin phosphoprotein repeat region and inherited defects of dentin. *Molecular genetics & genomic medicine* **4**: 28-38.

FIGURE LEGENDS

Figure 1 Pedigree, clinical photo, panoramic radiograph, sequencing chromatograms of family members and gene diagram of *DSPP*. (a) Pedigree of Family 1. (b) Clinical photo of the proband (III:3) at age 8 years. Remaining deciduous teeth show an amber-brown discoloration and mild to moderate attrition, but erupting permanent teeth look normal without any discoloration. (c) Panoramic radiograph of the proband at age 10 years reveals the characteristic thistle tube-shaped pulp chambers with pulp stones. (d) Clinical photo of the proband of family 3 at age 9 years 8 months. Remaining deciduous teeth exhibit a dark brown discoloration and severe attrition. Permanent dentition is normal in shape and color in most teeth, but the lingual surfaces of the maxillary anterior teeth show a mild brown hue at the cervical area. (e) Sanger Sequencing chromatograms of the mutations identified. Wild type (wt) and mutant (mut) nucleotide sequences are written on the above each chromatogram. The location of the mutations (deletion and insertion) was indicated with a red arrow in each chromatogram. Sequences based on the reference sequence for mRNA (NM_014208.3), where the A of the ATG translation initiation codon is nucleotide 1. (f) *DSPP* consists of 5 exons. Boxes indicate exons and the amino acids numbers encoded by the exon are shown below each exon. The white area indicates the non-coding part and blue area indicates DSP region. Orange color indicates DPP region (463-1301 amino acids). The area associated with DD-II is shown as a green double arrow. The red arrows indicate the position of identified mutations in this study.



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