

RESEARCH ARTICLE

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# A novel *DSPP* mutation causes dentinogenesis imperfecta type II in a large Mongolian family

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## Abstract

**Background:** Several studies have shown that the clinical phenotypes of dentinogenesis imperfecta type II (DGI-II) may be caused by mutations in *dentin sialophosphoprotein* (*DSPP*). However, no previous studies have documented the clinical phenotype and genetic basis of DGI-II in a Mongolian family from China.

**Methods:** We identified a large five-generation Mongolian family from China with DGI-II, comprising 64 living family members of whom 22 were affected. Linkage analysis of five polymorphic markers flanking *DSPP* gene was used to genotype the families and to construct the haplotypes of these families. All five *DSPP* exons including the intron-exon boundaries were PCR-amplified and sequenced in 48 members of this large family.

**Results:** All affected individuals showed discoloration and severe attrition of their teeth, with obliterated pulp chambers and without progressive high frequency hearing loss or skeletal abnormalities. No recombination was found at five polymorphic markers flanking *DSPP* in the family. Direct DNA sequencing identified a novel A→G transition mutation adjacent to the donor splicing site within intron 3 in all affected individuals but not in the unaffected family members and 50 unrelated Mongolian individuals.

**Conclusion:** This study identified a novel mutation (IVS3+3A→G) in *DSPP*, which caused DGI-II in a large Mongolian family. This expands the spectrum of mutations leading to DGI-II.

## Background

Dentinogenesis imperfecta type II (DGI-II) (OMIM # 125490) is an autosomal dominant dental disorder with a complete penetrance that affects both the primary and the permanent teeth [1]. DGI-II is characterized by amber and opalescent teeth, abnormal dentine leading to obliteration of the pulp chamber, and enamel that, although unaffected, tends to fracture. This causes the dentine to undergo rapid attrition, leading to a marked shortening of the teeth. The gene *DSPP* is located in the 6.6-cM D4S2691-D4S2692 interval at 4q21 and encodes a precursor protein, which is cleaved to yield dentine sialoprotein (DSP) and dentine phosphoprotein (DPP) [2-4]. A nonsense mutation in *DSPP* has been reported to cause DGI-II in a Chinese family [5] and other *DSPP* mutations have subsequently been demonstrated in

Chinese families with DGI-II [6-9]. In addition, families with DGI-II in other countries have been reported with mutations in *DSPP* [10-15]. However, the genetic basis of DGI-II in Mongolian families has not been explored before. In the present study, we describe a large, five-generation Mongolian family with DGI-II and report a novel *DSPP* mutation in this family.

## Methods

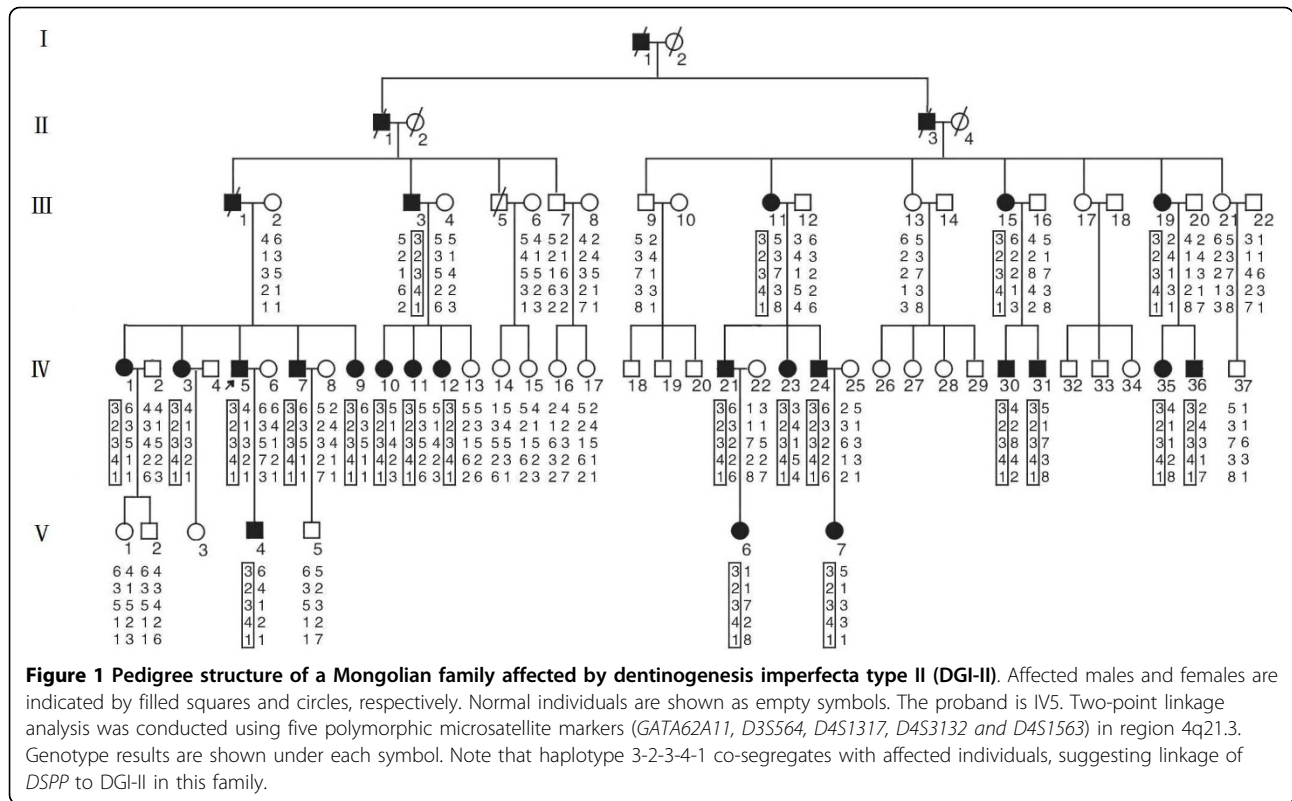
### Patients

We identified a large, five-generation Mongolian family with DGI-II consisting of 64 living family members, of which 22 were affected (Figure 1). All living members were examined clinically and taken for panoramic dental tomograms. The clinical and radiographic images were published under the patients' written permission. The study "Gene Research on Dentinogenesis Imperfecta in Mongolian Families" was approved by the Research Ethics Committee of Peking Union Medical College.

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### DNA extraction

Peripheral blood leukocytes were collected from 48 of the 64 family members, and human genomic DNA was extracted by using phenol - chloroform followed by ethanol precipitation.

### Genetic linkage and haplotype analysis

Two-point linkage analysis was conducted using five polymorphic markers (*GATA62A11*, *D3S564*, *D4S1317*, *D4S3132* and *D4S1563*) at 4q21.3. LOD scores were calculated using the MLINK program of the LINKAGE package. The parameters used for linkage analysis were autosomal dominant inheritance, complete penetrance, a mutation rate of zero, equal male-female recombination rates, equal allele frequency, and a disease allele frequency of 1 in 10,000.

### Sequence analysis of DSPP

Mutation screening was carried out using direct DNA sequence analysis. The exons of the *DSPP* gene were amplified by primers flanking the exon-intron boundaries (Table 1). Exon 4 was amplified into two, and Exon 5 was amplified into six fragments. PCR conditions for exons 1-5 were as following: a 5-min initial denaturation at 94°C, 35 cycles of 1- min denaturation at 94°C, 1- min annealing at 58°C, 58°C, 50°C, 60°C, 60°C, 60°C, 64°C, 60°C, 60°C, 55°C, and 55°C, respectively,

and a 1-min extension at 72°C, and a 5-min final extension at 72°C. PCR product were sequenced by Beijing AuGCT Biotechnology Co., Ltd <http://http:www.augct.com>.

We determined the sequences of all five exons and the exon flanking sequences of *DSPP* from 48 of affected and unaffected individuals in this family. The mutaton sites of 50 unrelated healthy Mongolian controls also were sequenced directly.

### Prediction of the mutation effect

In order to investigate whether the mutation will affect the splice donor site of exon 3, we used the BDGP site [http://www.fruitfly.org/seq\\_tools/splice.html](http://www.fruitfly.org/seq_tools/splice.html) to predicte the effect of gene mutation on the splicing site of the *DSPP*[16].

### Results

In the five-generation Mongolian family with DGI-II, the proband was a man aged 32 (Figure 1, IV5 ). His permanent teeth showed a shade of brown and almost complete attrition of the enamel layer without a history of periapical infections. All affected individuals showed discoloration and severe attrition of their teeth with obliterated pulp chambers. In addition, the enamel, although unaffected, had tended to fracture, causing the dentine to undergo rapid attrition, leading to a marked

**Table 1 Primers used for amplification and sequencing of the DSPP gene**

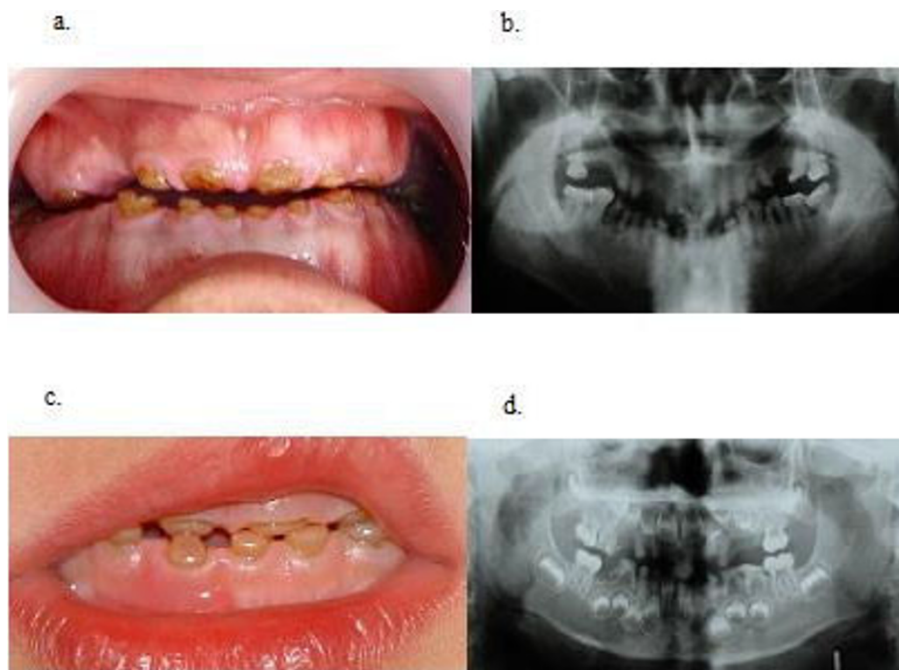
Exon	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Annealing Temperature (°C)
1	TCACCAAGTGAAGGAAGTGG	AAAGCCCAAGGTGGATTTT	58°C
2	GATGCCCCATAACACACC	CTCCATGACTTCTGGGCATT	58°C
3	AAGAACCTTTTCAATAGCCAGT	TGGAGAAGTTAATGGAATGTAGCAAC	50°C
4-1	TGCAATTTGCTTTCCTTCAAG	TGTTATTGCTTCCAGCTACTTGAG	60°C
4-2	CAATGAGGATGTCGCTGTTG	TGCCATTGAAAGAAATCAGC	60°C
5-1	TTCTTTCCTCCATCCTCCATAG	TGTCATCATTCCCATTGTTACC	60°C
5-2	CAAAAGGAGCAGAAGATGATGAC	TTGCTGCTGTCTGACTTGCT	64°C
5-3	CAAATCAGACAGTGGCAAAGGTAAT	CACTGCTATTGCTGCTGCTGTTGCT	60°C
5-4	GACAGCAGTAATAGTAACAGCAGCG	GCTGCTGCTGCTATTGCTATCACTG	60°C
5-5	GCAGTGACAGCAACGAAAGCAGCAAT	GTTGTACC GTTACCAGACTGCTC	55°C
5-6	TGACAGCACATCTGACAGCAAT	TCCCCAGTTGTTTTGTTT	55°C

shortening of the teeth. Both the primary and the permanent teeth were affected (Figure 2). No high-frequency hearing loss or obvious skeletal abnormalities were found in any of the affected individuals.

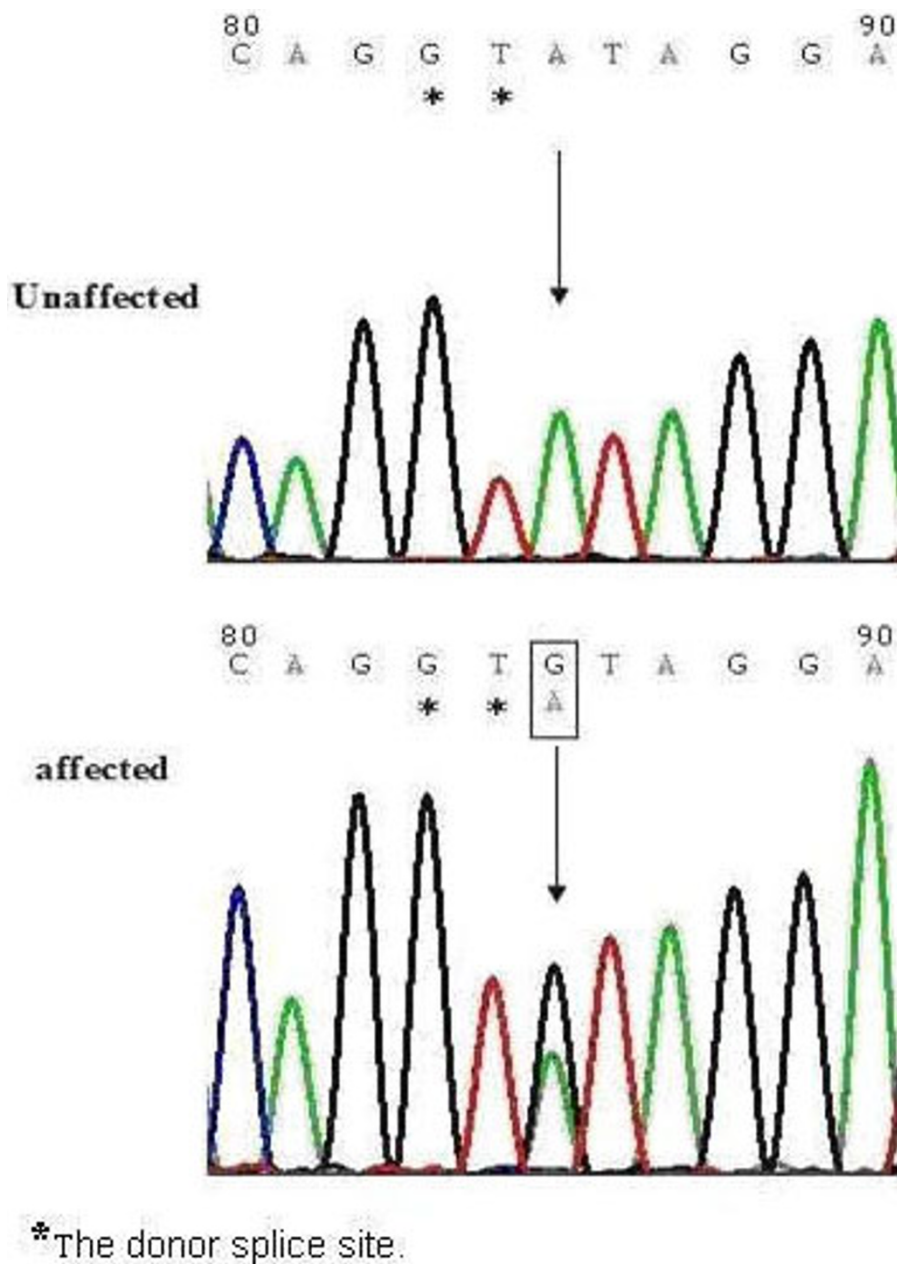
Through linkage analysis we obtained a maximal LOD score of 6.06 for marker D3S564 at  $\theta = 0.00$ , thereby demonstrating definitive linkage. Haplotype analysis showed that haplotype 3-2-3-4-1 cosegregated with the disease in this family, indicating that the disease locus

was linked to the chromosome region harboring *DSPP*, and that *DSPP* was a candidate gene (Figure 1).

Mutation screening showed a novel, functional A→G transition mutation adjacent to the donor splicing site (GT) within intron 3 of *DSPP* in all affected individuals, whereas this mutation was not found among the unaffected individuals in the family (Figure 3). Furthermore, we did not find this mutation in 50 unrelated, healthy Mongolian controls.



**Figure 2 Clinical analysis of dentinogenesis imperfecta type II (DGI-II).** The proband (IV5) is a man aged 32. His permanent teeth showed a shade of brown and almost complete attrition of the enamel layer without a history of periapical infections (a and b). Dentition of the 5-year-old son of the proband. His primary teeth had shown normal timing of eruption, but shortly thereafter become brownish and small due to cracking of the enamel and attrition of dentin. At the time of examination, his first permanent molars had just emerged and still showed an intact enamel (c and d).



**Figure 3 Identification of a novel mutation.** An A→G transition adjacent to the donor splicing site (GT) within intron 3 of *DSPP* was detected in all affected individuals, whereas this mutation was not detected in unaffected individuals of the DGI-II Mongolian family or in unrelated healthy Mongolian controls. DNA sequences for a normal family member (upper panel) and the proband IV5 (lower panel).

The available splicing site prediction software, the BDGP site, was utilized to predict the consequence of the mutation (IVS3+3A→G) in *DSPP*, the splice donor site of exon3 went from a score of 0.89 to <0.

### Discussion

We identified a novel mutation (IVS3+3A→G) in *DSPP* in a large Mongolian family suffering from dentinogenesis imperfecta II (DGI-II). This novel mutation (IVS3

+3A→G) resulted in a donor splicing site change from wild-type GTAT to mutated GTGT in one of the two *DSPP* alleles that co-segregate in affected individuals. This mutation did not exist in unaffected family members or in an additional 50 healthy Mongolian controls. These results suggest that the A→G mutation caused DGI-II in this Mongolian family.

DGI-II is a clinically heterogeneous disorder caused by *DSPP* mutations [7,17-19]. Previous studies have

reported DGI-II families with a mis-sense mutation in exon 2 [6], a nonsense mutation in exon 3 [5], splicing site mutations in intron 3+1 [6,9] and a frameshift mutation in intron 2 [9]. However, the molecular mechanisms by which *DSPP* mutations cause DGI-II are still unclear. In this Mongolian family, we speculate that the novel mutation is likely to produce a new splicing site and destroy the original splicing site within intron 3. This mutation may result in the abnormal intron splicing and lead to exon-skipping with a loss of exon 3, which encodes part of dentin sialoprotein protein. Because tissue samples from this family were unavailable, we were unable to prepare mRNA from the affected individuals to determine the sequences of *DSPP* transcripts.

To our knowledge, this study is the first report of a novel *DSPP* mutation causing DGI-II in a Mongolian family from China. This mutation differs from those found previously in other Chinese families and in families of other ethnic groups. Mongolians represent one of the major ethnic minority groups in China. They reside on the Inner Mongolian grassland in the north-east of China, where they live a nomadic lifestyle. This Mongolian family, whose forebears lived on the Horqin grassland in the eastern part of Inner Mongolia for many generations, is a relatively homogeneous population with characteristics that are advantageous for genetic research, including low divorce rate, limited mobility, consistent dietary habits and favorable environmental factors.

## Conclusion

This study documents a novel A→G transition mutation adjacent to the donor splicing site (GT) within intron 3 of *DSPP* that causes DGI-II in a large Mongolian family. This expands the spectrum of mutations that cause DGI-II.

## Abbreviations

DGI: dentinogenesis imperfecta; DPP: dentine phosphoprotein; DSP: dentine sialoprotein; DSPP: dentin sialophosphoprotein

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## Authors' contributions

HB designed the study and family recruitment, performed the linkage analysis, drafted the manuscript and obtained funding. HA supervised the study design. QW conducted clinical diagnoses and family recruitment. JM supervised the study and family recruitment. CQ did the genetic design, supervised the study and obtained funding. All other authors provided technical assistance, and all authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

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## References

1. Witkop CJ: Hereditary defects in enamel and dentine. *Acta Genet Stat Med* 1957, **7**(1):236-239.
2. Crosby AH, Scherpbier-Heddema T, Wijmenga C, Altherr MR, Murray JC, Buetow KH, Dixon MJ: Genetic mapping of the dentinogenesis imperfecta type II locus. *Am J Hum Genet* 1995, **57**(4):832-839.
3. MacDougall M: Refined mapping of the human dentine sialophosphoprotein (DSPP) gene within the critical dentinogenesis imperfecta type II and dentine dysplasia type II loci. *Eur J Oral Sci* 1998, **106**(Suppl 1):227-233.
4. MacDougall M, Simmons D, Luan X, Nydegger J, Feng J, Gu TT: Dentin phosphoprotein and dentin sialoprotein are cleavage products expressed from a single transcript coded by a gene on human chromosome 4. Dentine phosphoprotein DNA sequence determination. *J Biol Chem* 1997, **272**(2):835-842.
5. Zhang X, Zhao J, Li C, Gao S, Qiu C, Liu P, Wu G, Qiang B, Lo WH, Shen Y: DSPP mutation in dentinogenesis imperfecta Shields type II. *Nat Genet* 2001, **27**(2):151-152.
6. 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: Dentinogenesis imperfecta 1 with or without progressive hearing loss is associated with distinct mutations in DSPP. *Nat Genet* 2001, **27**(2):201-204.
7. Song Y, Wang C, Peng B, Ye X, Zhao G, Fan M, Fu Q, Bian Z: Phenotypes and genotypes in 2 DGI families with different DSPP mutations. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006, **102**(3):360-374.
8. Zhang X, Chen L, Liu J, Zhao Z, Qu E, Wang X, Chang W, Xu C, Wang QK, Liu M: A novel DSPP mutation is associated with type II dentinogenesis imperfecta in a Chinese family. *BMC Med Genet* 2007, **8**:52.
9. Wang H, Hou Y, Cui Y, Huang Y, Shi Y, Xia X, Lu H, Wang Y, Li X: A novel splice site mutation in the dentin sialophosphoprotein gene in a Chinese family with dentinogenesis imperfecta type II. *Mutat Res* 2009, **662**(1-2):22-27.
10. Rajpar MH, Koch MJ, Davies RM, Mellody KT, Kieley CM, Dixon MJ: Mutation of the signal peptide region of the bicistronic gene DSPP affects translocation to the endoplasmic reticulum and results in defective dentine biomineralization. *Hum Mol Genet* 2002, **11**(21):2559-2565.
11. Kim JW, Nam SH, Jang KT, Lee SH, Kim CC, Hahn SH, Hu JC, Simmer JP: A novel splice acceptor mutation in the DSPP gene causing dentinogenesis imperfecta type II. *Hum Genet* 2004, **115**(3):248-254.
12. Malmgren B, Lindskog S, Elgadi A, Norgren S: Clinical, histopathologic, and genetic investigation in two large families with dentinogenesis imperfecta type II. *Hum Genet* 2004, **114**(5):491-498.
13. Kim JW, Hu JC, Lee JI, Moon SK, Kim YJ, Jang KT, Lee SH, Kim CC, Hahn SH, Simmer JP: Mutational hot spot in the DSPP gene causing dentinogenesis imperfecta type II. *Hum Genet* 2005, **116**(3):186-191.
14. Holappa H, Nieminen P, Tolva L, Lukinmaa PL, Alaluusua S: Splicing site mutations in dentine sialophosphoprotein causing dentinogenesis imperfecta type II. *Eur J Oral Sci* 2006, **114**(5):381-384.
15. Lee SK, Lee KE, Jeon D, Lee G, Lee H, Shin CU, Jung YJ, Lee SH, Hahn SH, Kim JW: A novel mutation in the DSPP gene associated with dentinogenesis imperfecta type II. *J Dent Res* 2009, **88**(1):51-55.
16. Reese MG, Eeckman FH, Kulp D, Haussler D: Improved Splice Site Detection in Genie. *J Comp Biol* 1997, **4**(3):311-23.
17. MacDougall M, Dong J, Acevedo AC: Molecular basis of human dentine diseases. *Am J Med Genet A* 2006, **140**(23):2536-2546.

18. Kim JW, Simmer JP: **Hereditary dentine defects.** *J Dent Res* 2007, **86**(5):392-399.
19. Acevedo AC, Santos LJ, Paula LM, Dong J, MacDougall M: **Phenotype characterization and DSPP mutational analysis of three Brazilian dentinogenesis imperfecta type II families.** *Cells Tissues Organs* 2009, **189**(1-4):230-236.

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