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, 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. 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 mutation 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 to predict 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 dentin to undergo rapid attrition, leading to a marked
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 θ = 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). Mutations 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.

| Exon | Forward primer sequence (5′–3′) | Reverse primer sequence (5′–3′) | Annealing Temperature (°C) |
|------|---------------------------------|---------------------------------|---------------------------|
| 1    | TCACCAAGTGAAGGAAGTGG            | AAAGCCCAAGGTGGATTITT           | 58°C                      |
| 2    | GATGCCCCCTAATACCACACC          | CTCCATGACCTCTGGGCATT          | 58°C                      |
| 3    | AAGACCTTTTCAATAGGCAGT         | TGGAAGTATATGGGAATGACAC         | 50°C                      |
| 4-1  | TGCATTGTGCTTCTTCAAG            | TGGTATGCTTCAGCTACTTGA         | 60°C                      |
| 4-2  | CAATGAGGATGTCGCTTGTG           | TGCCATGAAAGAATCAAGC           | 60°C                      |
| 5-1  | TTTCTTCCCTCATCCTCCCATAG       | TGTCATCAATCCCCATGTTACC        | 60°C                      |
| 5-2  | CAAAAGGACAGCAAGATGATGAC        | TTGTCGCTGTGCTGACTTGGC         | 64°C                      |
| 5-3  | CAATCACAGACAGTGCCAAGTAAT       | CACTGCTATTGCTGCTGCTGCTGCT     | 60°C                      |
| 5-4  | GACAGACGATGATGACAGCAGCGG       | GCTGTCCGCTGCTGCTGCTGCTGCT     | 60°C                      |
| 5-5  | GCAGTGACAGCAACGAAACAGCAGAAT  | GTTGTACGCGGATACAGCATTGCTC     | 55°C                      |
| 5-6  | TGACAGCAGCATCTGACAGCAAT       | TCCCCCAGTGTGTTTTGGTTT         | 55°C                      |

**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).
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 northeast 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
DSPP: dentin phosphoprotein; DPP: dentine phosphoprotein; DS: dentin sialoprotein; DSM: DSPP: dentin sialoprotein phosphoprotein

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