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Phenotypic features of dentinogenesis imperfecta associated with osteogenesis imperfecta and *COL1A2* mutations

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Objective. Dentinogenesis imperfecta (DI) requires dental treatment. This study investigated the characteristics of DI teeth associated with osteogenesis imperfecta (OI) and *COL1A2* mutations.

Study Design. Whole exome and Sanger sequencing were performed. Three primary teeth (called "OIDI teeth") obtained from 3 unrelated *COL1A2* patients were investigated and compared with 9 control teeth from age-matched healthy individuals using colorimetry, micro–computed tomography, Knoop microhardness, energy dispersive X-ray spectroscopy, scanning electron microscopy, and histology.

Results. All patients were identified with heterozygous glycine substitutions in *COL1A2*. The *COL1A2* mutations, c.1531G>T and c.2027G>T, were *de novo*, whereas c.3106G>C was inherited. OIDI1, 2, and 3 teeth had a substantial decrease in dentin microhardness and lightness. OIDI2 enamel microhardness was significantly reduced, whereas OIDI1 and 3 had enamel microhardness comparable to that of control individuals. The OIDI1 pulp cavity was large; OIDI2 was narrow; and OIDI3 was obliterated. OIDI1 and 3 had significantly higher carbon levels than those in control individuals. Numerous ectopic calcified masses, sparse and obstructed dentinal tubules, dentin holes, and collagen disorientation were observed.

Conclusions. OIDI teeth had reduced lightness and variable pulp morphology. Weak dentin, mineral disproportion, and abnormal ultrastructure could contribute to the brittleness of OIDI teeth and adhesive restoration failure. Here, we expand the phenotypic spectrum of *COL1A2* mutations and raise awareness among dentists seeing patients with OI. (Oral Surg Oral Med Oral Pathol Oral Radiol 2021;000:4544)

Dentinogenesis imperfecta (DI) is a genetic disorder of dentin affecting the primary and permanent dentition. The Shields classification, based on phenotypes, proposed dividing DI into 3 types.[1] Shields DI type I is associated with osteogenesis imperfecta (OI), whereas DI types II and III are nonsyndromic. Due to the availability of genetic data concerning the causes of inherited dentin disorders, it has been proposed that the pathogenesis of Shields DI I is different from that of DI II and DI III and should be considered as a unique entity.[2,3]

Shields DI II (OMIM accession no. 125490) and DI III (OMIM accession no. 125500) are associated with mutations in the dentin sialophosphoprotein (*DSPP*) gene (MIM*125485).[4-6] Recent genetic studies have shown that Shields DI II and DI III could be the same disease with phenotypic variability.[3,5] In contrast, Shields DI I is primarily caused by heterozygous mutations in type I collagen coding gene *COL1A1* or *COL1A2* (MIM*120150 or MIM*120160, respectively).[7-9] It was suggested that the abnormal dentin associated with OI should be called "DI-like" or opalescent teeth associated with OI.[2,3]

Statement of Clinical Relevance

Dentinogenesis imperfecta, osteogenesis imperfecta, and *COL1A2* mutations lead to a significant decrease in dentin hardness and lightness, mineral disproportion, pulp obliteration, and abnormal dentin, including holes, ectopic calcified masses, sparse tubules, and collagen disorientation. Dentists should be aware of these anomalies.

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Clinically, DI teeth are opalescent, brittle, and prone to fracture.[10-13] Bulbous crowns, cervical constriction, and pulp obliteration are radiologic features. There are a few studies showing the ultrastructural features of DI teeth, such as weak dentin, irregular tubules, and changes in mineral content. However, these phenotypes concerned *DSPP*-associated DI and the opalescent teeth associated with OI caused by *COL1A1* mutation.[4,14-19] Only one previous study demonstrated the dentin defects in a patient with *COL1A2* mutation. However, that patient had a glycine substitution in the carboxyl terminus of *COL1A2* leading to OI/Ehlers-Danlos overlap syndrome, which is not typical of OI.[20] Because of the limited understanding of the dental phenotypic spectrum related to *COL1A2* mutations, the present study collected primary teeth (called OIDI) from 3 unrelated patients with OI who had glycine substitutions in *COL1A2*. Our aims were to thoroughly investigate and expand knowledge of the ultrastructural, physical, and mechanical properties of OIDI teeth associated with *COL1A2* mutations.

MATERIALS AND METHODS

Subject enrollment

Three unrelated patients with OI and DI and their family members were recruited. Informed consent was obtained from all participants or their parents/legal guardians. The project was approved by the research ethics committee at the Faculty of Dentistry, Chulalongkorn University, Thailand (HREC-DCU 2018-092), and was conducted in accordance with the Declaration of Helsinki and additional requirements.

Mutation identification

Peripheral blood samples were collected and extracted to obtain genomic DNA. Whole-exome sequencing (WES) was performed at Macrogen Inc. (Seoul, Korea) using a HiSeq 4000 sequencer (Illumina) and processed as previously described.[21-23] The variants were filtered out if they were noncoding variants or synonymous exonic variants; if they were not in the genes associated with OI;[24,25] or if they were found in >1% in the 1000 Genomes Project database, Genome Aggregation Database (Gnomad.broadinstitute.org), or in-house database of 2166 Thai exomes. The identified mutations were validated by Sanger sequencing using primers shown in Supplemental Table S1.

Tooth samples

Three primary teeth were obtained from 3 unrelated patients with OI with *COL1A2* mutations. OIDI1 was the primary right first molar obtained from patient 1 (Pt-1); OIDI2 was the primary left central incisor from Pt-2; and OIDI3 was the primary right second molar from Pt-3. Each OIDI tooth was studied and compared with 3 tooth-type matched controls collected from age-matched healthy individuals.

Tooth color

Tooth color was measured on the buccal surface of each tooth 3 times using a digital intraoral colorimeter (VITA Easyshade V; VITA Zahnfabrik H. Rauter GmbH & Co KG, Bad Sackingen, Germany). The L*a*b values were recorded according to the International Commission on Illumination. The L* displays the lightness in the black-and-white scale; a* is the saturation in the red-and-green scale; and b* is the saturation in the blue-and-yellow scale. The color difference (ΔE*) was calculated using the equation $\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$.

Micro–computed tomography (micro-CT)

The whole tooth was scanned using micro-CT (Specimen μCT35; SCANCO Medical AG, Brüttisellen, Switzerland). A square area of 16 × 16 and 104 × 104 pixels was created in 30 sections of the sample. Mineral density was measured using the Image Processing Language (SCANCO Medical AG, Brüttisellen, Switzerland).

Microhardness

After micro-CT scanning, the tooth was fixed in a resin block. The block was cut longitudinally into buccal and lingual segments. Microhardness was measured on the lingual segment using a Knoop microhardness tester (FM700 e Type D; Future Tech, Kanagawa, Japan) at 100 gF for 15 s/load. Thirty locations on the enamel and dentin were selected. The Knoop microhardness (H_K) values were calculated according to the formula $H_K = 14\,229\,P/L^2$ (*P,* applied test load [gF]; *L,* indentation diagonal length [μm]).

Mineral composition, ultrastructure, and histology

Mineral composition, comprising carbon (C), oxygen (O), phosphorus (P), and calcium (Ca), was measured at 3 spots on the buccal segment using energy dispersive X-ray spectroscopy (EDX) (ISIS 300 EDX System; Oxford Instruments, Abingdon, UK). Tooth ultrastructure was analyzed using a scanning electron microscope (SEM) (JSM 5410-LV; Jeol Ltd., Tokyo, Japan). The segment was then embedded in paraffin, sectioned, and stained with hematoxylin and eosin and Masson's trichrome.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows software (version 22; IBM Corp., Armonk, NY, USA). Data normality was assessed by using the Kolmogorov-Smirnov test. For

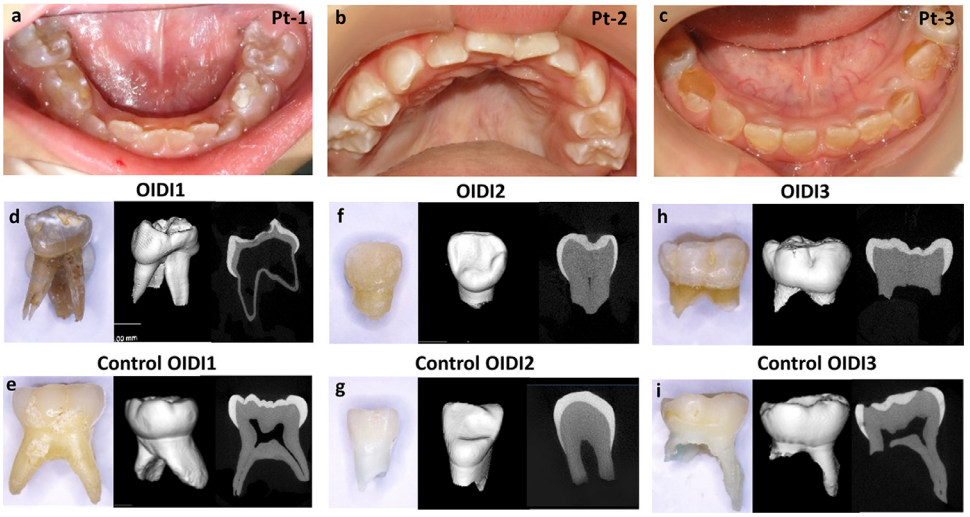

Fig. 1. Phenotypes of Patients 1, 2 and 3 and OIDI1, 2, and 3. (a-c) Intraoral image of Pt-1, 2, and 3 show opalescent primary teeth. (d-i) Light microscopic image, three-dimensional reconstruction, and longitudinal cross-section of the OIDI teeth and controls.

normally distributed data, independent group comparison was analyzed using analysis of variance followed by Tukey's test for pairwise comparison. For non-normally distributed data, the Kruskal-Wallis test was used for independent group comparison, and the Mann-Whitney *U* test was used for pairwise comparison. The significance level was set at .05.

RESULTS

Medical features and genetic mutations

Three unrelated patients were diagnosed with OI and DI on the basis of clinical, radiographic, and laboratory examinations. Pt-1 was 7 years old; Pt-2 was 6 years old; and Pt-3 was 13 years old. All 3 patients had short stature, bone curvature, low bone mineral density, flat feet, DI, and a history of bone fractures (Figure 1a−c). The phenotype of Pt-1 was described in our previous study.[24] All 3 patients had received intravenous pamidronate therapy (7.2 mg/kg) every 2 months.

Using WES, we identified Pt-1 with a *de novo* heterozygous missense mutation, c.1531G>T (p.Gly511 Cys), in exon 26 of *COL1A2* (NM_000089.4).[24] Pt-1's parents were healthy and had normal teeth. Pt-2 possessed a heterozygous missense mutation, c.3106G>C (p.Gly1036 Arg), in exon 47 of *COL1A2*. The mutation was inherited from the mother, who

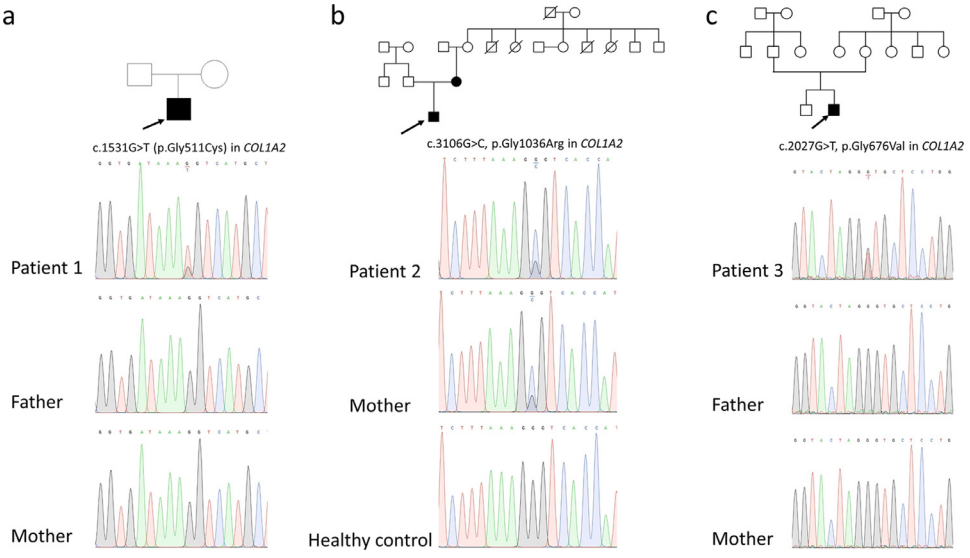

Fig. 2. Family pedigree and chromatogram of patients and their families. A filled symbol indicates an individual with dentinogenesis imperfecta associated with osteogenesis imperfecta. Arrows indicate the probands.

also had OI and DI. Pt-3 harbored a *de novo* heterozygous missense mutation, c.2027G>T (p.Gly676 Val), in exon 34 of *COL1A2*. Pt-3's parents were healthy and had normal teeth. All 3 mutations were glycine substitutions in *COL1A2* (Figure 2). These mutations were predicted to be pathogenic according to the American College of Medical Genetics and Genomics standards and guidelines.[26] Genetic findings confirmed the diagnosis of OI in Pt-1, -2, and -3.

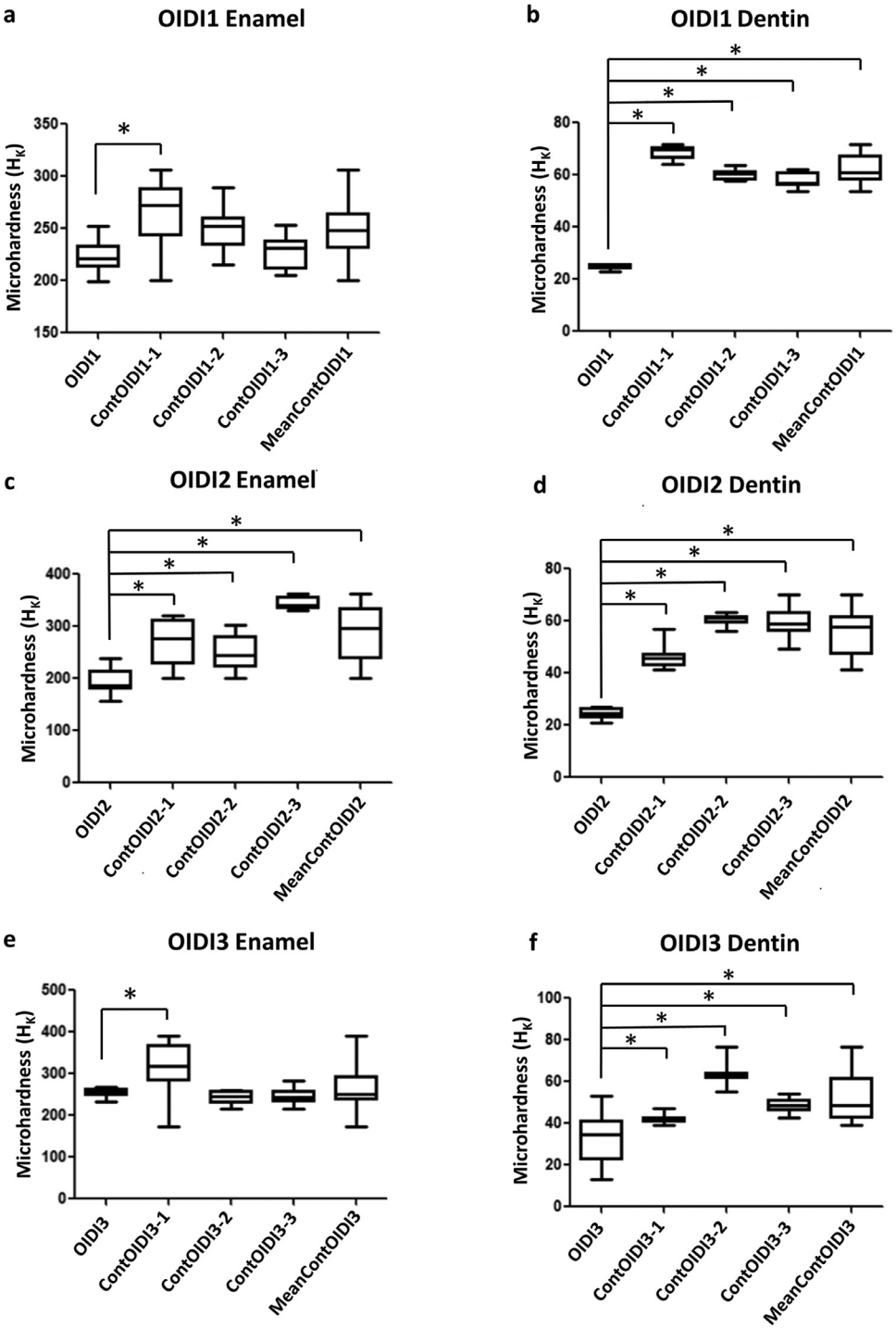

Fig. 3. Knoop microhardness of enamel and dentin of OIDI1, 2, 3 and controls. Dentin microhardness values of OIDI1, 2, and 3 are significantly lower than controls. The microhardness values of OIDI1 and OIDI3 enamel are comparable to controls, while that of OIDI2 enamel is lower than controls. *Significant at $P < 0.05$. Cont, control.

Dental phenotype

Primary tooth samples, OIDI1, OIDI2, and OIDI3, were collected from Pt-1, 2, and 3, respectively. Clinically, the OIDI teeth were opalescent. The lightness (L*) values of OIDI1, OIDI2, and OIDI3 (38, 68, and 62 respectively) were lower than their controls, revealing that the OIDI teeth were darker than normal teeth. On the basis of a* and b* values, OIDI1 was bluer and greener than controls; OIDI2 was yellower and redder than controls; and OIDI3 was bluer and redder than controls. The color differences (ΔE) between OIDI1, OIDI2, and OIDI3 and their controls were 50.62, 25.70, and 28.00, respectively.[27] These values indicate that the color distinction between the OIDI and normal teeth is perceivable by the human eye (Supplemental Table S2).

Micro-CT cross-sections demonstrated that OIDI1 had a very wide pulp cavity, whereas that of OIDI2 was narrow and that of OIDI3 was obliterated (Figure 1d−i). The enamel and dentin mineral densities of OIDI1, 2, and 3 analyzed by micro-CT were comparable to those of controls (Supplemental Table S3).

The Knoop microhardness values of OIDI1, OIDI2, and OIDI3 dentin were significantly lower than those of controls. The enamel microhardness values of OIDI1 and OIDI3 were comparable to those of controls, whereas OIDI2 had a significantly lower microhardness than that of controls (Figure 3 and Supplemental Table S4).

The mineral content was measured by EDX. In dentin, OIDI1 had a significantly increased level of carbon and significantly reduced levels of phosphate and calcium compared with controls. A significant increase in carbon was observed in OIDI2 dentin, whereas a significant decrease in carbon content was observed in OIDI3 dentin compared with that of controls. The OIDI1 and OIDI3 enamel had significantly higher levels of carbon, but lower levels of oxygen, phosphorus, and calcium, than those of controls. No significant differences in mineral content were observed in the

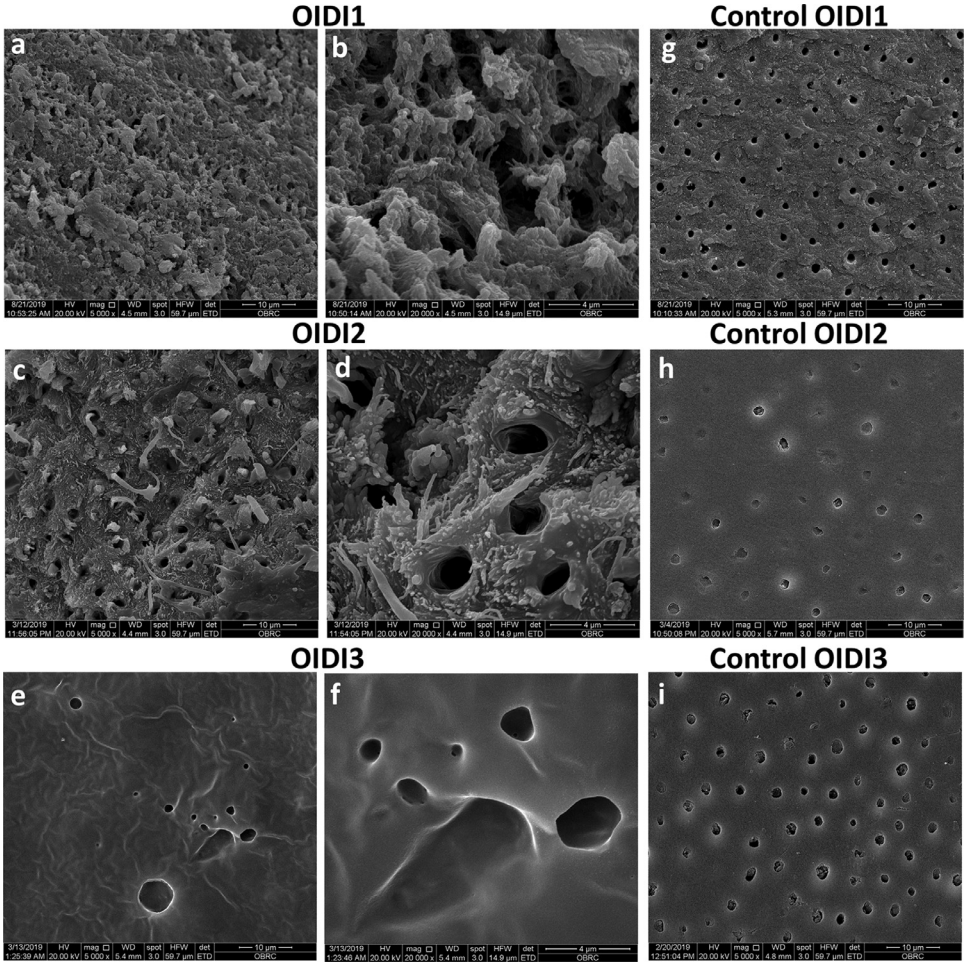

Fig. 4. Scanning electron microscopic images of OIDI1, 2, 3 and controls. (a-f) OIDI1 and OIDI2 have a rough dentin texture comprising large gaps and numerous ectopic calcified masses. OIDI3 has a wavy dentin surface and holes. (g-i) Controls exhibit the typical arrangement of dentinal tubules. a, c, e, g, h, i: magnification 5,000x; b, d, f: 20,000x.

OIDI2 enamel compared with controls (Supplemental Table S5 and Supplemental Figure S1).

SEM demonstrated that the OIDI teeth had severe dentin abnormalities. These teeth lacked a uniform arrangement of dentinal tubules. OIDI1 and OIDI2 presented a rough dentin texture containing large gaps and ectopic, calcified masses, whereas OIDI3 dentin showed a wavy surface and large holes. Some dentinal tubules were observed in OIDI1 and OIDI2; however, they were minimal in OIDI3. In controls, the tubule orientation was regular, and the dentin surface was smooth (Figure 4). Histologically, regular alignment of dentinal tubules and collagen fibers was found in controls, whereas disorientated and sparse tubules and collagen fibers were present in the OIDI teeth (Figure 5).

DISCUSSION

In this study, 3 unrelated patients diagnosed with OI and DI were identified with glycine substitutions in *COL1A2*. Type I collagen is a major component of bone and tooth extracellular matrix. Mutations in the glycine triple-helix of collagen chains have been demonstrated to cause OI and DI in a large number of OI cases.[7,11,24,28-30] The prevalence of DI was increased when the mutations occurred between p.Gly376 and p.Gly745 and from p.Gly982 to the amino-terminal end of the triple-helical domain.[31] Correspondingly, Pt-1, Pt-2, and Pt-3 had DI features and possessed the mutations p.Gly511 Cys, p.Gly1036 Arg, and p.Gly676 Val, respectively.

Clinically, all 3 patients had opalescent teeth that were darker than normal, which was perceivable by the human eye. Based on the L*a*b values, the discoloration of the OIDI teeth observed in this study was consistent with a previous report demonstrating that patients with OI had a tooth color in the gray/blue-yellow/brown range.[32] Micro-CT scanning revealed that OIDI teeth had a variable pulp size ranging from very wide to obliterated, and OIDI1 had the widest pulp cavity and the thinnest dentin. It is possible that the very thin dentin of OIDI1 could make the teeth weak and prone to deterioration and fracture. The absence or obliteration of the OIDI2 and OIDI3 pulp cavities could be due to excessive formation of reparative dentin to compensate for rapid attrition.[16,17,33]

Significant decreases in dentin hardness were consistently observed in OIDI teeth. Similarly, reduced dentin hardness was reported in patients with DI Shields type I and *COL1A1* mutations and in those with DI Shields type II.[18,34] The mineral content of OIDI1, 2, and 3 was diversely different from that of controls. Disproportionate mineral composition was reported in DI Shields type II teeth.[35] At the ultrastructural level, SEM revealed that the OIDI teeth had multiple dentin abnormalities, including disorganized and reduced dentinal tubules; rough dentin texture; large holes; and ectopic, calcified masses. These features overlapped with those previously reported.[36-38] Histologically, the collagen fibers in OIDI teeth were irregular in size and arrangement, as well as reduced in number. It was postulated that the dentinal tubules in DI teeth were

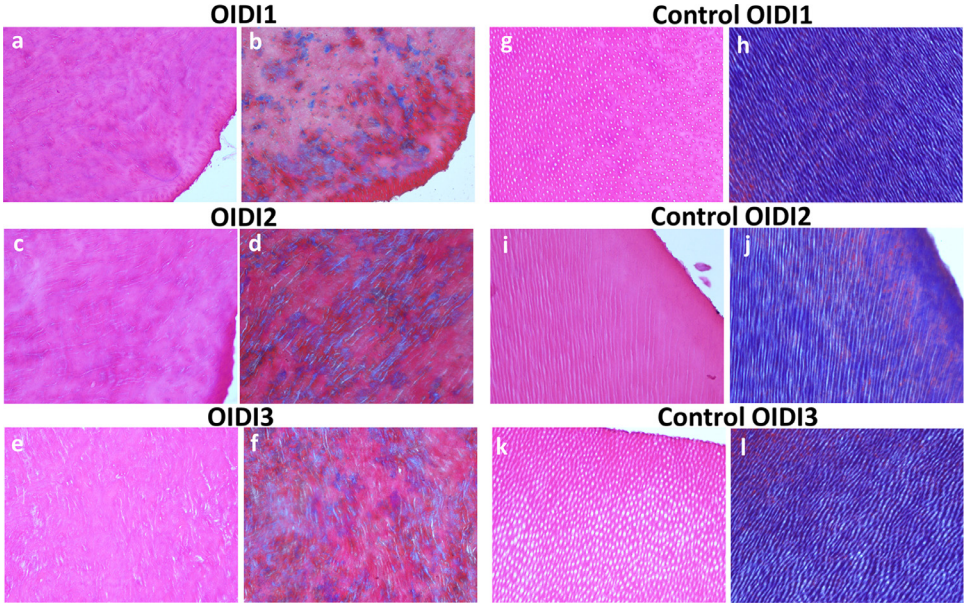

Fig. 5. Histological images of OIDI1, 2, 3 and controls. (a-f) OIDI dentin has disorientated and sparse dentinal tubules and collagen fibers. (g-l) Controls exhibit regular alignment of tubules and collagen fibers. a, c, ,e, g, i, k: hematoxylin and eosin staining; b, d, f, h, j, l: Masson's trichrome staining. Microscopic magnification at 40x.

occluded by excessive production of peritubular dentin.[17,36] Future studies using tooth samples from patients with OI at different ages would verify these findings. The abnormal formation of dentinal tubules and collagen fibers could limit the penetration of dental adhesives and formation of a hybrid layer, affecting the quality of a dental restoration.[39,40]

To conclude, this study expands the phenotypic features, including the ultrastructural, physical, and mechanical properties, of OIDI teeth associated with glycine substitutions in *COL1A2*. The OIDI teeth had a distinctly dark color, variable pulp morphology, reduced dentin hardness, disproportionate mineral composition, and dentin ultrastructural defects. These findings provide insights into the accumulated dentin defects of OIDI that could contribute to tooth weakness and failure of adhesive restorations and raise awareness among dentists providing dental care for these patients.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.oooo.2021.01.003.

REFERENCES

1. Shields ED, Bixler D, el-Kafrawy AM. A proposed classification for heritable human dentine defects with a description of a new entity. *Arch Oral Biol*. 1973;18:543-553.
2. Chetty M, Roberts T, Stephen LXG, Beighton P. Hereditary dentine dysplasias: terminology in the context of osteogenesis imperfecta. *Br Dent J*. 2016;221:727-730.
3. de La Dure-Molla M, Philippe Fournier B, Berdal A. Isolated dentinogenesis imperfecta and dentin dysplasia: revision of the classification. *Eur J Hum Genet*. 2015;23:445-451.
4. Porntaveetus T, Osathanon T, Nowwarote N, et al. Dental properties, ultrastructure, and pulp cells associated with a novel DSPP mutation. *Oral Dis*. 2018;24:619-627.
5. Barron MJ, McDonnell ST, Mackie I, Dixon MJ. Hereditary dentine disorders: dentinogenesis imperfecta and dentine dysplasia. *Orphanet J Rare Dis*. 2008;3:31.
6. Maciejewska I, Chomik E. Hereditary dentine diseases resulting from mutations in DSPP gene. *J Dent*. 2012;40:542-548.
7. Marini JC, Forlino A, Bächinger HP, et al. Osteogenesis imperfecta. *Nat Rev Dis Primers*. 2017;3:17052.
8. van Dijk FS, Cobben JM, Kariminejad A, et al. Osteogenesis imperfecta: a review with clinical examples. *Mol Syndromol*. 2011;2:1-20.
9. Tauer JT, Robinson ME, Rauch F. Osteogenesis imperfecta: new perspectives from clinical and translational research. *JBMR Plus*. 2019;3:e10174.
10. Kim JW, Simmer JP. Hereditary dentin defects. *J Dent Res*. 2007;86:392-399.
11. Udomchaiprasertkul W, Kuptanon C, Porntaveetus T, Shotelersuk V. A family with homozygous and heterozygous p.Gly337Ser mutations in *COL1A2*. *Eur J Med Genet*. 2020;63:103896.
12. Tongkobpetch S, Limpaphayom N, Sangsin A, Porntaveetus T, Suphapeetiporn K, Shotelersuk V. A novel de novo *COL1A1* mutation in a Thai boy with osteogenesis imperfecta born to consanguineous parents. *Genet Mol Biol*. 2017;40:763-767.
13. Porntaveetus T, Nowwarote N, Osathanon T, et al. Compromised alveolar bone cells in a patient with dentinogenesis imperfecta caused by *DSPP* mutation. *Clin Oral Investig*. 2019;23:303-313.
14. Leal CT, Martins LD, Verli FD, de Souza MA, Ramos-Jorge ML. Case report: clinical, histological and ultrastructural characterization of type II dentinogenesis imperfecta. *Eur Arch Paediatr Dent*. 2010;11:306-309.
15. Majorana A, Bardellini E, Brunelli PC, Lacaita M, Cazzolla AP, Favia G. Dentinogenesis imperfecta in children with osteogenesis imperfecta: a clinical and ultrastructural study. *Int J Paediatr Dent*. 2010;20:112-118.
16. Sapir S, Shapira J. Dentinogenesis imperfecta: an early treatment strategy. *Pediatr Dent*. 2001;23:232-237.
17. Turkkahraman H, Galindo F, Tulu US, Helms JA. A novel hypothesis based on clinical, radiological, and histological data to explain the dentinogenesis imperfecta type II phenotype. *Connect Tissue Res*. 2020;61:526-536.
18. Ibrahim S, Strange AP, Aguayo S, et al. Phenotypic properties of collagen in dentinogenesis imperfecta associated with osteogenesis imperfecta. *Int J Nanomed*. 2019;14:9423-9435.
19. Malmgren B, Lindskog S. Assessment of dysplastic dentin in osteogenesis imperfecta and dentinogenesis imperfecta. *Acta Odontol Scand*. 2003;61:72-80.
20. Budsamongkol T, Intarak N, Theerapanon T, Yodsanga S, Porntaveetus T, Shotelersuk V. A novel mutation in *COL1A2* leads to osteogenesis imperfecta/Ehlers-Danlos overlap syndrome with brachydactyly. *Genes Dis*. 2019;6:138-146.
21. Intarak N, Theerapanon T, Thaweesapphithak S, Suphapeetiporn K, Porntaveetus T, Shotelersuk V. Genotype-phenotype correlation and expansion of orodental anomalies in LTBP3-related disorders. *Mol Genet Genom*. 2019;294:773-787.
22. Nitayavardhana I, Theerapanon T, Srichomthong C, et al. Four novel mutations of *FAM20A* in amelogenesis imperfecta type IG and review of literature for its genotype and phenotype spectra. *Mol Genet Genom*. 2020;295:923-931.
23. Porntaveetus T, Abid MF, Theerapanon T, et al. Expanding the oro-dental and mutational spectra of Kabuki syndrome and expression of *KMT2D* and *KDM6A* in human tooth germs. *Int J Biol Sci*. 2018;14:381-389.
24. Hemwong N, Phokaew C, Srichomthong C, et al. A patient with combined pituitary hormone deficiency and osteogenesis imperfecta associated with mutations in *LHX4* and *COL1A2*. *J Adv Res*. 2020;21:121-127.
25. Rossi V, Lee B, Marom R. Osteogenesis imperfecta: advancements in genetics and treatment. *Curr Opin Pediatr*. 2019;31:708-715.
26. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405-424.

27. Yamanel K, Caglar A, Özcan M, Gulsah K, Bagis B. Assessment of color parameters of composite resin shade guides using digital imaging versus colorimeter. *J Esthet Restor Dent*. 2010;22:379-388.
28. Zhytnik L, Maasalu K, Duy BH, et al. De novo and inherited pathogenic variants in collagen-related osteogenesis imperfecta. *Mol Genet Genom Med*. 2019;7:e559.
29. Lu Y, Zhang S, Wang Y, Ren X, Han J. Molecular mechanisms and clinical manifestations of rare genetic disorders associated with type I collagen. *Intractable Rare Dis Res*. 2019;8:98-107.
30. Basel D, Steiner RD. Osteogenesis imperfecta: recent findings shed new light on this once well-understood condition. *Genet Med*. 2009;11:375-385.
31. Rauch F, Lalic L, Roughley P, Glorieux FH. Genotype-phenotype correlations in nonlethal osteogenesis imperfecta caused by mutations in the helical domain of collagen type I. *Eur J Hum Genet*. 2010;18:642-647.
32. Andersson K, Dahllof G, Lindahl K, et al. Mutations in *COL1A1* and *COL1A2* and dental aberrations in children and adolescents with osteogenesis imperfecta – a retrospective cohort study. *PLoS One*. 2017;12:e0176466.
33. Singhal P, Arya S, Vengal M, Bhalodia M. Dentinogenesis imperfecta type II – a case report with review of literature. *Glob J Med Res*. 2014;14:25-28.
34. Wieczorek A, Loster J, Ryniewicz W, Ryniewicz AM. Dentinogenesis imperfecta - hardness and Young's modulus of teeth. *Acta Bioeng Biomech*. 2013;15:65-69.
35. Wieczorek A, Loster J. Analysis of the mineral composition of permanent teeth in dentinogenesis imperfecta type II. *J Stomatol*. 2012;65:404-410.
36. Hall RK, Maniere MC, Palamara J, Hemmerle J. Odontoblast dysfunction in osteogenesis imperfecta: an LM, SEM, and ultrastructural study. *Connect Tissue Res*. 2002;43:401-405.
37. Lygidakis NA, Smith R, Oulis CJ. Scanning electron microscopy of teeth in osteogenesis imperfecta type I. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1996;81:567-572.
38. Kantaputra PN, Sirirungruangsarn Y, Intachai W, Ngamphiw C, Tongsima S, Dejkhamron P. Osteogenesis imperfecta with ectopic mineralizations in dentin and cementum and a *COL1A2* mutation. *J Hum Genet*. 2018;63:811-820.
39. Guo J, Wang L, Zhu J, Yang J, Zhu H. Impact of dentinal tubule orientation on dentin bond strength. *Curr Med Sci*. 2018;38:721-726.
40. Schüpbach P, Krejci I, Lutz F. Dentin bonding: effect of tubule orientation on hybrid-layer formation. *Eur J Oral Sci*. 1997;105:344-352.

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