

Ultrastructural Characteristics of Dental Hard Tissues Associated with Osteogenesis Imperfecta

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Abstract

Objectives: The aim of this study was to investigate the physical properties including surface roughness, hardness, elastic modulus, and ultrastructural appearance of dental hard tissues including the enamel, coronal dentin, and root dentin associated with syndromic dentinogenesis imperfecta (DGI). **Methods:** Patient included in this study was diagnosed as affected by osteogenesis imperfecta (OI) and DGI (syndromic DGI). Clinical and radiographic oral examinations were performed. Characteristics of the tooth affected with DGI (DGI tooth) including surface roughness, hardness, elastic modulus, and ultrastructural appearance were studied compared to the controls. **Results:** The patient was diagnosed with OI type IV and DGI. Physical examinations indicated classic signs of OI including bone deformities, curved extremities, short stature, and DGI. Oro-dental phenotypes of the patient showed brownish opalescent teeth with bulbous crown, cervical constriction, pulp obliteration, tooth deterioration, and malocclusion. Analyses of DGI tooth showed that the hardness of the enamel, coronal dentin, and root dentin was significantly reduced. The elastic modulus of DGI's enamel and root dentin was significantly decreased compared to that of the controls. The coronal dentin of DGI tooth displayed a markedly rough surface with the reduction in number and irregular appearance of dentinal tubules. The root dentin was disorganized with scattered of ectopic mineralization, irregular pattern and variable diameter of dentinal tubules. The dentinoenamel junction was irregular. The surface roughness of DGI's enamel was not decreased. **Conclusions:** The syndromic DGI tooth demonstrated significant changes in the hardness, elastic modulus, and formation of dentin and DEJ. These indicate that the tooth is malformed and weak which could negatively affect tooth integrity and adhesion property of dental restorations. Our study expands the understanding of pathologic changes of dental hard tissues associated with syndromic DGI and raises awareness of dental practitioner for the management of DGI patients.

Key words: Osteogenesis imperfecta, skeletal dysplasia, surface roughness, nanohardness, scanning electron microscopy

Introduction

Osteogenesis imperfecta (OI), also called “bristle bone disease”, is a heterogeneous group of genetic disorders affecting connective tissue which is mainly characterized by increased susceptibility to fractures. The prevalence of OI ranges from 1 in 5,000 - 20,000 live births.¹ The primary cause of OI is associated with heterozygous mutations in the *COL1A1* and *COL1A2* genes which encode the alpha 1 and alpha 2 chains of collagen type I.² The recessive mutations associated with genes encoding proteins involved in collagen type I biogenesis, other than *COL1A1* and *COL1A2*, are shown to cause more severe OI.³

The classification of OI is traditionally defined into four common types (type I-IV) based on clinical findings and inheritance pattern by Sillence in 1979.⁴ Each type of OI shares common clinical characteristics such as bone fragility, skeletal deformities, and joint hyperlaxity which may differ in severity. The most common and mildest form is OI type I with specifically presence of blue sclerae and hearing impairment which can be observed over a half of patients. OI type II is the most severe form characterized by multiple skeletal fractures and severe deformities which mostly result in perinatal lethality. Type III usually involves a newborn presenting with severe progressive skeletal deformities, short stature and high mortality rate in early life. Type IV can be differentiated from the other aforementioned types by the absence of blue sclerae. The patient with this type of OI often has recurrent fractures and variable degrees of deformity of long bones and spine. The OI type V with moderate to severe bone fragility, characterized by presence of progressive calcification of the inter-osseous membranes in the forearms and legs, was suggested to be an additional type of OI classification.^{5,6} Clinically, OI can be categorized into mild, moderate, severe, and

extremely severe type based on its pre- and postnatal severity. These emphasize the importance of phenotypic characterization for diagnosis, classification, and severity assessment of the disease.³

The clinical manifestations of OI range from rarely recognized symptoms to perinatal mortality. Apart from bone deformities, other presentations of OI include blue sclerae, skeletal deformities, hearing loss, and DGI.³ Oral manifestations in OI patients have been reported to include class III malocclusion, posterior crossbite, and generalized severe attrition.⁷ The common radiographic features observed in OI include osteopenia, multiple bone fractures, and bone deformities. The skull shows multiple wormian bones in the sutures.⁸

DGI is one of the associated clinical features frequently found in OI which can affect both primary and permanent dentitions. The presence of DGI in OI patients has been suggested to be particularly related to severe physical manifestations.³ The incidence of DGI is 1 in 8,000.⁹ Clinically, the crown presents severe attrition of enamel with dentin exposed and change in color varies from yellow to opalescent gray or brown. Since collagen type I is the main component in dentin, the weak underlying dentin renders the teeth prone to rapid wear and fracture. Radiographically, the DGI teeth have bulbous crowns with constricted short roots in both dentitions. Pulp chambers may be abnormally wide but become obliterated over a period of time. Histologically, the dentino-enamel junction is malformed. The dentinal tubules are coarse, branched, and reduced in number.¹⁰ DGI is widely classified into three types (type I - III). DGI type I is associated with OI, called syndromic DGI, and caused by mutation in *COL1A1* and *COL1A2* genes.¹¹ Type II is phenotypically similar to type I but without OI wherein mutations in the dentin sialophosphoprotein gene (*DSPP*) is responsible for the pathology.^{12,13} DGI type

III is rare and only found in the triracial Brandywine population of Maryland, USA.

The characteristics of OI and DGI are extensively diverse and have not been fully investigated especially tooth defects associated with syndromic DGI. Majority of previous reports mainly focused on dentin defects via scanning electron microscope (SEM) which showed highly variable features.¹⁴⁻¹⁸ In addition, the evidence showed that enamel was possibly affected by underlying dentin anomalies.¹⁶ The measurement of surface roughness of the crown, a parameter used to quantify the micro-structure of enamel surface,¹⁹ will be useful to represent consequences of dentin alteration. Furthermore, altered physical properties of the teeth including hardness and elastic modulus have been shown to affect adhesion of dental restorations.²⁰⁻²² To date, the information on physical properties of both enamel and dentin of DGI teeth is very limited.²³ Therefore, the present study aimed to investigate the physical properties including surface roughness, hardness, elastic modulus, and ultrastructural appearance of dental hard tissues including the enamel, coronal dentin, and root dentin associated with syndromic DGI. Our findings will expand the understanding of DGI which will be of assistance for clinicians to deliver appropriate dental management in affected patients.

Materials and Methods

Subject enrollment

A patient included in this study was diagnosed as affected by OI and DGI at the Genetic Clinics, Chulalongkorn Hospital and referred to the Faculty of Dentistry, Chulalongkorn University for oro-dental management. The diagnoses of the syndromes were based on clinical, radiographic, and laboratory findings. Extraoral and intraoral photographs were taken. Clinical and radiographic oral examinations were performed at the Fac-

ulty of Dentistry, Chulalongkorn University. The study was approved by the research ethics committee at Faculty of Dentistry, Chulalongkorn University. Informed written consent was obtained from the patient. The patient's maxillary left second molar (called DGI tooth in this study) was extracted due to poor prognosis. Two second molars were used as controls. They were collected from healthy individuals who had similar ages, and did not have any systemic diseases or medications affecting tooth and bone structures. After extraction, the teeth were placed in 10% formalin solution and stored in saline solution.

Surface roughness measurement

The surface roughness was studied on the lingual surface using a surface Profilometer (Talyscan 150, Taylor Hobson Ltd., UK). Each specimen was measured 30 times randomly at every 600 micrometer distance on Y-axis at the stylus speed of 1000 micrometer/second. Tracing area was 2 x 2 mm and cut-off length was 0.025 mm. The calculation of the surface topography parameters was carried out with the TalyMap Universal program. The same areas of the DGI and control samples were selected.

Tooth sectioning

Samples were embedded in acrylic block with sticky wax and divided along bucco-lingual plane parallel to the tooth axis using the slow-speed precision saw (Isomet 1,000 Precision Saw, Buehler, Lake Bluff, IL, USA) with diamond disc at a speed of 450 rpm under constant water cooling. Then, a cut surface was grinded with Grit#1000 silicon-carbide paper under water irrigation and polished with alumina powder on polishing pad (10-inch MICROPAD, Pace technologies) until the surface was flat and parallel. Once the samples were prepared,

micrographs were obtained by stereomicroscopy (Olympus SZ61, Tokyo, Japan).

Measurement of nanohardness and elastic modulus

The indentation experiment was performed using nano-base indentation system (Ultra Micro-Indentation System, UMIS II, CSIRO, Australia). The finished specimens were mounted on metal base with thin double side tape. The test was done under dry condition. Elastic modulus and hardness of specimens were measured with the calibrated diamond Berkovich (three-sided pyramid) indenter. All samples were indented at loading forces from 1 to 200 mN on 30 locations randomly. In all loading-unloading cycles, more than 20 indentations were performed at each load. The IBIS software was used to calculate values for elastic modulus and hardness as a function of penetration depth, hardness testing (ht), for each indentation. The DGI teeth and controls were analyzed at the same areas.

Scanning Electron Microscopy (SEM)

Samples were etched with 37% phosphoric acid, rinsed with water, dehydrated, dried, and coated with gold powder for 110 seconds. The scanning electron microscopy (QuantaFeg 250, FEI Company, Oregon, USA) was employed to examine the dental tissues of DGI teeth compared with those of the controls.

Statistical Analysis

The statistical analysis was performed with the GraphPad Prism 5 Software Package (GraphPad Software, Inc., San Diego, CA, USA). All data was analyzed by Mann Whitney U test and Independent T-Test (significance level at 0.05).

Results

A 43-year-old Thai male was referred to the Faculty of Dentistry, Chulalongkorn University for extraction of carious tooth. He was diagnosed with osteogenesis imperfecta at the King Chulalongkorn Memorial Hospital, Thailand. The patient had experienced multiple episodes of bone fractures. He spontaneously broke his ribs and clavicles at the age of 3 months and his right femur at age 7 years. At 25 years of age, he had a car accident causing the fracture of both his femurs. Physical examinations revealed short stature (156 cm; 25th percentile), marked limb deformities, and widened antero-posterior body axis (Fig. 1A, B). The deformed limbs were not tender or swollen. The patient did not have blue sclerae.

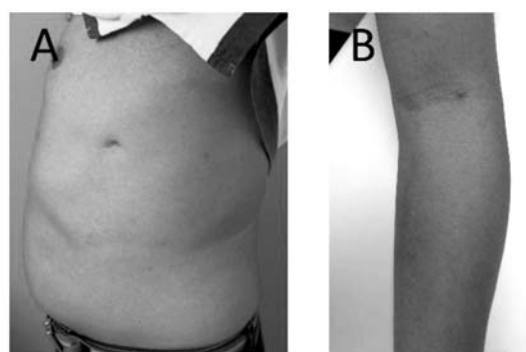


Fig. 1 Clinical features of the patient. A. The antero-posterior body axis was wide. B. The left arm showed marked deformities.

Radiographic examination revealed generalized osteopenia. His humerus and tibia were thin (Fig. 2A, B). The bone densitometry results concordantly showed reduced bone mineral density of lumbar spine (0.534 g/cm^2 ; z-score -3.0). The patient had never received bisphosphonate treatment.



Fig. 2 Skeletal radiographs of the patient. The extremities were narrow with reduced radiopacity. A. Right humerus B. Left tibia

Intraoral examination of the patient revealed yellowish brown and opalescent teeth, severe attrition, and malocclusion. The oral soft tissue was unremarkable (Fig. 3A). The maxillary left second and third molars were extracted due to carious reasons. The teeth had bulbous crown, amalgam filling, and curved root (Fig. 3B). Dental radiograph showed marked cervical constriction and obliteration of coronal and radicular pulp cavities (Fig. 3C). The maxillary left second molar had large cavity. The root of maxillary left third molar showed periapical radiolucency.

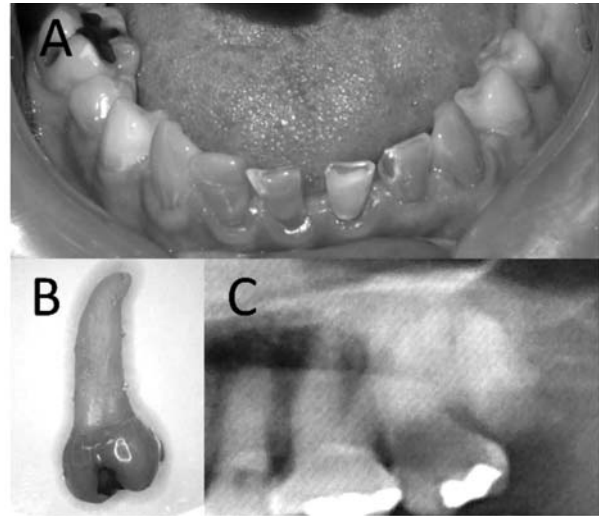


Fig. 3 Oro-dental manifestations of the patient. A. The mandibular teeth showed brown opalescent color with partial fracture. B. The maxillary left second molar showed bulbous crown with marked cervical constriction. C. Radiographically, the pulp cavities were obliterated and the roots were curved. Large cavity was observed on the maxillary left second molar.

Surface roughness measurement

In the enamel, the surface roughness analyses showed that the DGI tooth demonstrated a significantly lower roughness average (Ra) value than the controls ($p < 0.0001$) (Fig. 4).

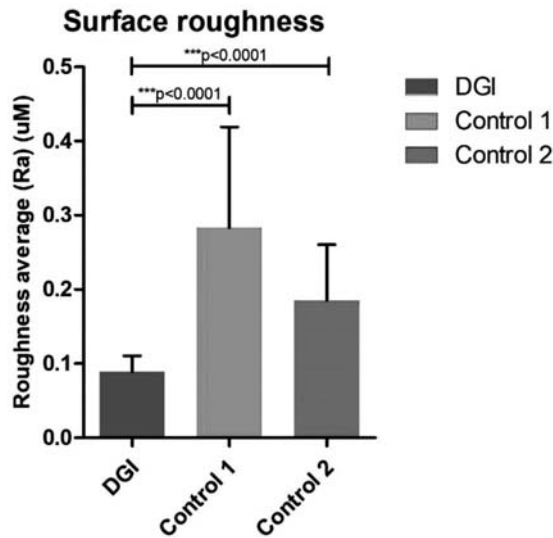


Fig.4 Bar graphs showed roughness average (Ra) values of DGI tooth compared to control 1 and 2. DGI tooth demonstrated the lowest value of surface roughness which was significantly different from the other controls.

Nanohardness and elastic modulus

The nanohardness of enamel, coronal dentin, and root dentin of DGI tooth was significantly lower than that of the controls (Fig. 5A). The difference in elastic modulus

between DGI and controls showed similar pattern to the hardness values except the coronal dentin which did not show a statistical significance (Fig. 5B).

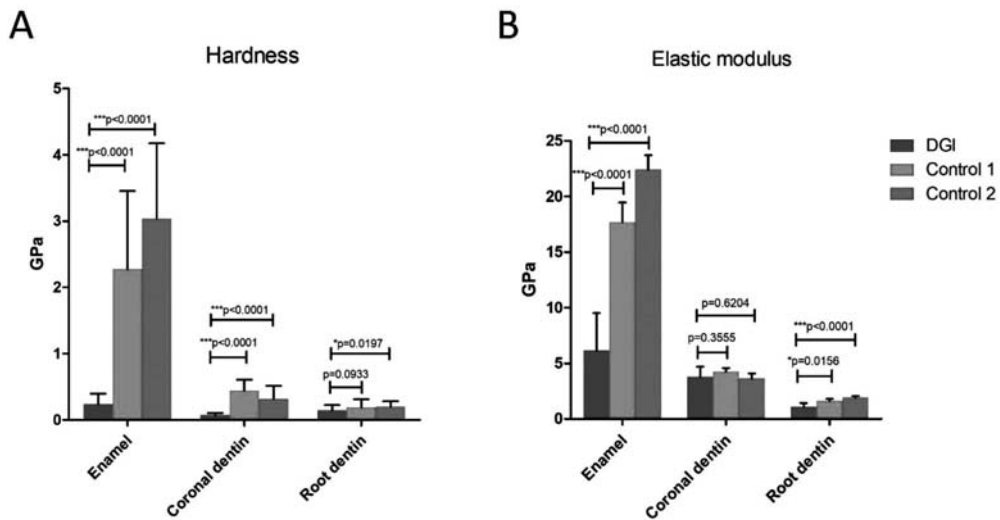


Fig.5 Bar graphs demonstrated the hardness (A) and elastic modulus (B) values of DGI tooth and controls. A. DGI tooth showed a significantly lower level of hardness in all three aspects, except at root dentin which was not significantly different from control 1's. B. The significant difference was noted at aspects of enamel and root dentin, whereas the difference at coronal dentin did not reach statistical significance.

Scanning Electron Microscopy (SEM)

In coronal dentin, DGI tooth displayed a markedly rough surface, reduced number of dentinal tubules, irregularly spaced tubules, and variable tubular diameter while the controls showed a typical pattern of regular dentinal tubules (Fig. 6A, B). At the dentinoenamel

junction, the DGI tooth demonstrated a notably rough surface of dentin with appearance of scattered mineral deposits (Fig. 6C, D). The dentin surface at the DGI root was disorganized containing scattered ectopic mineralization and dispersed dentinal tubules with variable diameters (Fig. 6E-H).

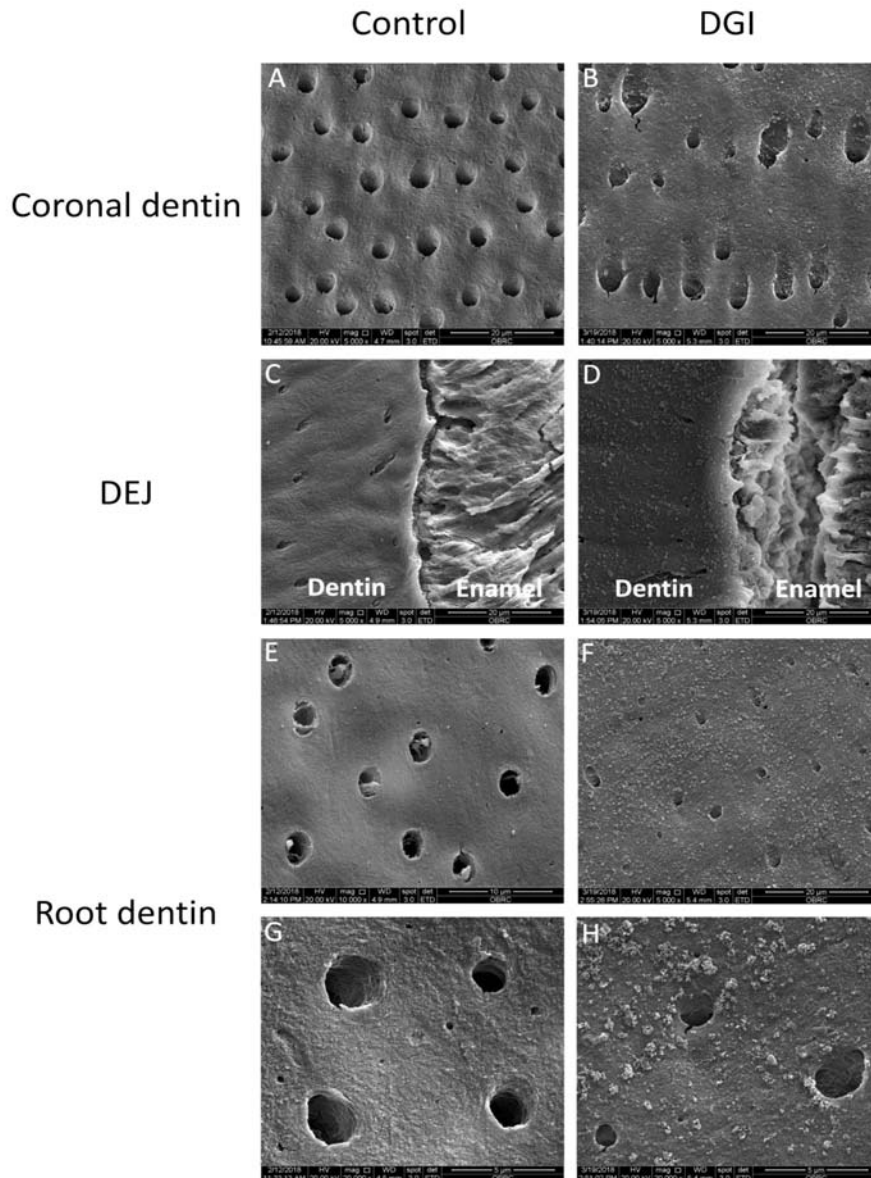


Fig. 6 Scanning electron microscopic findings of the DGI tooth compared to the controls. A, B. The coronal dentin of DGI tooth showed decreased and dispersed dentinal tubules compared to the typical pattern of tubules with uniform diameter. C, D. The DGI tooth showed rough DEJ. E-H. The root dentin of DGI tooth appeared rough containing ectopic mineralization and irregularly spaced tubules. (SEM original magnification x5,000 (E, F) and x20,000 (G, H))

Discussion

The present study demonstrates clinical and radiographic characteristics, ultrastructure, and physical properties of syndromic DGI. Based on the patient's physical features including mild skeletal severity without the presence of hearing loss and blue sclerae, the diagnosis of OI type IV was given.⁴ The majority of OI has been associated with mutations in *COL1A1* and *COL1A2* genes which encode polypeptide chains of type I collagen. Pathogenic variants in those genes have been shown to cause abnormalities in collagenous tissue including bone, skin, sclerae, and dentin.¹¹ Consistently, our patient obviously showed bone deformity and abnormally yellowish-gray and opalescent tooth, bulbous crowns, and pulp obliteration, suggesting DGI associated with OI.

The enamel of our DGI tooth showed smooth surface as its roughness value was less than the controls. However, this finding cannot be compared with previous studies since surface roughness of DGI teeth has never been reported.

The ultrastructures of coronal and root dentin of our syndromic DGI including rough surface with deviated presence of dentinal tubules tooth was consistent with previous reports. Lygidakis *et al.*¹⁴ reported that the main characteristics of DGI dentin were reduced in number, irregular pattern, and diameter of dentinal tubules, and rough surface of interdental space. We also observed scattered ectopic mineralization on the dentin surface of our DGI patient. This feature was first reported by Kantaputra *et al.*²⁴ They suggested that it was caused by copolymerization of normal and abnormal collagens and distortion of normal fibril morphology which could be responsible for tooth integrity.²⁴

In addition, our SEM showed that the DEJ of DGI tooth was irregular compared to the controls. The DEJ

of DGI teeth mostly demonstrated an irregular scalloped appearance interrupted with some areas of straight DEJ.^{25,26} Previous study showed that the rough DEJ could contribute to the detachment of enamel from underneath abnormal dentin.²⁷ These altered structures of dentin, DEJ, and enamel of DGI teeth indicate the high susceptibility of the affected teeth to deterioration.

We observed that the hardness of enamel, coronal dentin, and root dentin of DGI tooth were significantly lower than that of the controls and the elastic modulus of DGI enamel and root dentin were significantly reduced. Consistently, Wieczorek *et al.*²³ reported that the hardness of DGI teeth was seven times decreased and Young's modulus was six times reduced, compared to those of sound teeth.

Based on several altered characteristics of DGI tooth including ultrastructure and physical properties of the dentin, DEJ, and enamel, the teeth could be prone to rapid deterioration and develop dental pathology. These are consequences of malformed collagen and dentin mineralization.²⁸ Studies have shown that penetration of dental adhesives to create a hybrid layer and resin tags are important factors for successful restorations of tooth-colored filling materials.²² The defects in dentinal tubules could jeopardize the bonding of dental adhesive agents. Regular monitoring of dental restorations in DGI patient should therefore be implemented. Genetic analyses of the patient are currently being performed to elucidate disease-causing mutations. Future studies covering a large number of tooth samples obtained from DGI patients with identified pathogenic variants will provide solid data on physical, mechanical, and chemical phenotype correlated with genotype in DGI. These will expand understandings of the disease.

Conclusions

Our study demonstrates that the syndromic DGI teeth had defects in both ultrastructure and physical properties of dental hard tissues including the enamel, coronal dentin, and root dentin. These changes negatively affect tooth integrity and adhesion property of restorations. Importantly, the syndromic DGI patients suffer from both medical and oro-dental problems. Comprehensive management of the patient by medical and dental professionals should be properly arranged.

Acknowledgements

The study was supported by the Thailand Research Fund (TRF) (DPG6180001) and Office of Higher Education Commission (OHEC) Thailand (MRG6080001), the Chulalongkorn Academic Advancement into Its 2nd Century Project, and the 90TH Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund).

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