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Thickness and microhardness of deciduous tooth enamel with known *DLX3* mutation

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ABSTRACT

Aim: To investigate the thickness and hardness of teeth affected by a 2-bp deletion (c.561_562delCT) in the *DLX3* gene.

Methods and materials: Extracted maxillary deciduous second molar was collected from the affected individual at age 12 years 7 months. Samples were sectioned buccolingually after embedding in epoxy resin. We measured the enamel thickness and microhardness and performed an elemental analysis using an electron probe microanalyser.

Results: On average, the hardness of the enamel with a 2-bp deletion in *DLX3* was about 53% of normal enamel hardness. The mutant enamel thickness was about half of the thickness of the normal control. The calcium level in the enamel with the 2-bp deletion was slightly decreased, while the magnesium level was slightly increased, in comparison to levels measured for normal teeth.

Conclusion: This study shows that enamel affected by a 2-bp deletion in *DLX3* has reduced thickness as well as diminished microhardness. These data may explain the severe attrition and interdental spacing observed in affected individuals.

1. Introduction

The distal-less homeobox 3 (*DLX3*) gene, located on chromosome 17q21, is a homeobox gene with a crucial role during embryonic development.[1] It has been shown that a 4-bp deletion (c.571_574delGGGG) in this gene results in tricho-dento-osseous syndrome (TDO; OMIM 190320).[2] TDO syndrome is characterized by defects in hair, teeth and bone, with an autosomal dominant inheritance pattern.[1,3] The main clinical features include unique curly or kinky hair at birth that eventually straightens in many cases, enamel hypoplasia with taurodontism, and increased bone density.[4]

The case with c.561_562delCT in the *DLX3* gene reported by Dong et al. resulted in only enamel phenotype (hypoplastic-hypomaturation amelogenesis imperfecta with taurodontism, AIHHT; OMIM 104510) while the same mutation identified independently in two families of different ethnic background was responsible for the classic TDO.[5] Thus it has been debated whether this novel 2-bp deletion in the *DLX3* gene causes non-oral symptoms, such as defects in the hair, increased bone density or nail involvement.

Dental phenotypes such as taurodontism, enamel thickness and mineral content have been reported for the TDO syndrome associated with the 4-bp deletion in the *DLX3*

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gene,[3,6] but the tooth characteristics associated with the 2-bp deletion in *DLX3* have not yet been reported. Therefore, in this study, we measured the enamel thickness, microhardness and elemental content of a primary maxillary second molar with a 2-bp deletion in the *DLX3* gene.

2. Materials and methods

This experiment was undertaken with the understanding and written consent of the patient according to the Declaration of Helsinki. The study protocol was independently reviewed and approved by the Institution Review Board at the Seoul National University Dental Hospital.

2.1. Samples

A deciduous right maxillary second molar was collected from a male patient with a known *DLX3* mutation (c.561_562delCT) at age 12 years 7 months. Control teeth (deciduous maxillary second molars) were collected from normal controls of the same gender at similar age.

2.2. Microhardness

The deciduous tooth was embedded in the epoxy resin and cut using a rotary diamond saw. The tooth was then sectioned buccolingually at the mesiobuccal and distobuccal cusps. For this test, six control teeth were used. Samples were polished using 200, 600, 800, 1000 and 2000 grit SiC papers. The microhardness score was measured at six points (enamel; near surface, middle, near DEJ, dentin; near DEJ, middle, pulpal). The measurement of the microhardness was performed four times at each point. Vickers microhardness (VHN) was measured using a microhardness tester (HMV-2, Schimadzu, Japan) where a 4.903 N load was applied for 10 s to obtain the measurement.

2.3. Enamel thickness

Sections were carbon-coated using a carbon coater (CC7650, Quorum technologies, UK) and SEM image was taken at 30× magnification using a field emission scanning electron microscope (S-4700, Hitachi, Japan). For this test, six control teeth were used and the measurement was repeated five times. Maximal enamel thickness perpendicular to DEJ was measured using image J (version 1.41, NIH, USA) on the buccal and labial slopes excluding cuspal area due to attrition.

2.4. Electron probe microanalysis

Elemental microanalyses were performed using an Electron Probe Micro Analyzer (JXA-8900R, JEOL, Tokyo, Japan) equipped with Wavelength Dispersive X-ray Spectroscopy (WDS). The standards used for calibration were Apatite [$Ca_5(PO_4)_5(F,Cl)$], Indium phosphide (InP) and Periclase (MgO) for calcium, phosphate and magnesium, respectively. The counting time at each point was 20 s with a 1 μm diameter of the electron beam at 15.0 kV and 10 nA. For this test, three normal samples were used. Measurements were performed at six points (enamel; near surface, middle, near DEJ, dentin; near DEJ, middle, pulpal). Three-spot (about 50 μm distance) measurements were performed at each point.

3. Results

3.1. Microhardness

The microhardness value of the *DLX3* mutant enamel was very low compared to that of the normal enamel (less than 95% confidence interval). On average, the hardness of the *DLX3* mutant enamel was about 53% of the hardness of normal enamel. However, the microhardness value of the *DLX3* mutant dentin was similar to that of normal dentin (Fig. 1).

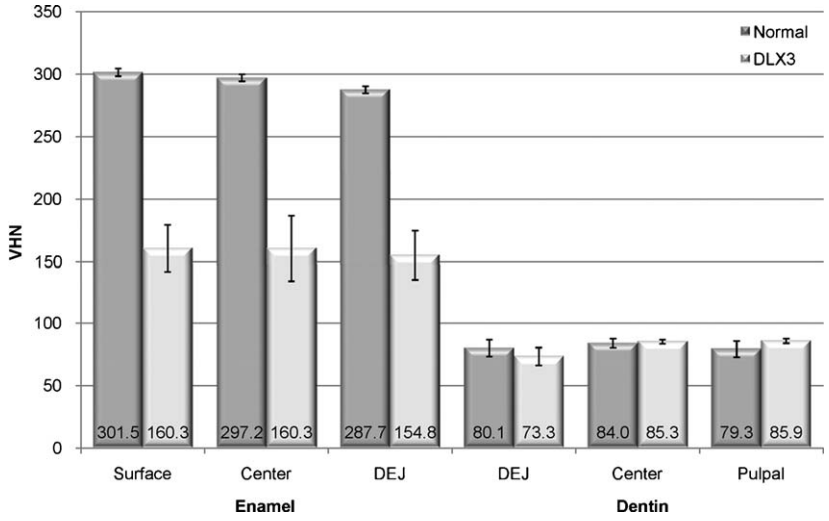

Fig. 1 – Microhardness of normal and affected teeth. The Vickers microhardness number (VHN) is indicated in the box. The 95% confidence interval is indicated in the box for the normal teeth. Standard deviation is indicated in the box for the 2-bp mutant DLX3 teeth.