Evaluation of Dens Evaginatus by CBCT and Exome sequencing in Thai Population

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Abstract

Dens evaginatus (DE) is a dental developmental anomaly with reports of variability in the frequency of the trait between racial groups and familial occurrences. This study aims to investigate characteristics of dens evaginatus and associated genomic variants. DNA samples of four patients with dens evaginatus and their fathers and mothers were taken and whole exome sequencing was performed. For radiographic analysis, ten teeth with dens evaginatus from six patients were examined and received CBCT. Depending on the clinical diagnosis of pulpal and periapical status, all teeth received either prophylaxis treatment or endodontic treatment. Participants were categorized as the exposed pulp group or the non-exposed pulp group. 3D images were evaluated with one volume viewer. Factors including main pulp canal extension, dens evaginatus-located cusp steepness, size, shape, location and pulp extension of dens evaginatus cusp were tested for association with clinical pulp exposure using Pearson’s chi-square or Fisher’s Exact Test. From the results, genes of genetic interest to 18 genes were narrowed down but a definite pathogenic variant was not identified in all the participants. From the CBCT investigation, there was a significantly higher chance of pulpal exposure in a tooth with dens evaginatus with a ratio of less than 0.45 and degree of buccal cusp inclination of less than 33°. Size, shape and location of dens evaginatus cusp are not statistically related to pulpal exposure. In conclusion, factors influencing clinical pulpal exposure in a tooth with DE were main pulp canal extension and dens evaginatus-located cusp steepness (p<0.05).

Keywords: Dens evaginatus, Exome sequencing, Genetic inheritance, Pulp exposure

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Introduction

Dens evaginatus (DE) is a dental developmental anomaly exhibited by an extra cusp or tubercle on the occlusal surface of teeth. It is commonly found on the premolars, but sometimes on the molars and incisors. The DE contains enamel, dentinal core, and usually pulpal tissue. According to the Oehler’s classification in 1967, 90% of teeth with DE had pulpal extension within the extra cusp.¹ Due to its protuberance, DE is susceptible to wear or fracture due to trauma. Once the extending pulp is exposed to the oral environment, the oral bacteria
may evade the pulp cavity causing infection. Many caries-free premolars have been reported with periapical pathology due to a fracture of DE.

The observation of DE is variable among racial groups. It is predominantly observed in the Asian population with an incidence ranging from 0.5 to 4.3%. According to the previous study examining 5,696 patients at Chiang Mai University, an incidence of 1.01% was reported in the Thai population. Previous literatures have suggested that DE is a multifactorial etiology, resulted from both genetic and environmental factors. The finding of DE inherited in many members of the same family suggests genetic influence. To date, available data on genetic variants associated with DE is still limited.

Several classifications of DE have been proposed, based on anatomical shape, location, or pulp content. Lau et al., 1955 classified the tubercle or DE into four anatomical shapes: smooth, grooved, terraced, and ridged. Merrill et al., 1964 categorized DE into a drop, nipple, pointed cone, or cylindrical cone. Oehlerls et al., 1967 classified DE by the histologic appearance of pulp horn inside the tubercle, comprising wide, narrow, or constricted pulp horn, isolated pulp horn remnant, and no pulp horn. Reichart and Tontiniron, 1975 divided DE by the location into two types: the tubercle arising from occlusal inclined plane and the tubercle located in the center of the occlusal surface. Small invagination or pinpoint cavity type was also observed that could be the result of DE attrition or fracture, leading to exposure of dentin and dental pulp.

However, none of these classifications correlates clinical findings with radiographic features. They are not applicable for clinical management and widely used by dentists. A lack of understanding of the correlation between clinical and radiographic features of DE results in improper diagnosis, prognosis, and management of DE.

Therefore, the aim of this study was to investigate the characteristics of DE and DE-associated genomic variants and investigate the correlation between clinical appearances, CBCT images, and pulpal extension of DE.

Materials and Methods

Participants

This study was approved by the Ethics Committee, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand (HREC-DCU 2019-059). For genetic investigation, four families, each consisting of a member with a dental record with DE, their father and mother, were recruited for examination and blood/saliva sampling. After the dental examination, we realized that the participants had received either prophylaxis treatment or endodontic treatment on their DE teeth and were unable to participate in the CBCT investigation. Therefore, a new group of participants were recruited for the CBCT investigation with the following inclusion criteria: 1. diagnosis of DE 2. DE tooth has not received any dental treatment. Six participants, comprising two males and four females at 10-42 years of age with 10 teeth with DE (four participants had two teeth with DE, two participants had one tooth with DE) were recruited. Informed consent was obtained from each participant.

Genetic investigation

Blood samples were submitted by each enrolled patient, the patient’s father and mother. 3 mL of blood was collected in a blood collection tube with EDTA by the technician. For patients who did not agree to having their blood drawn, they were asked to collect 2 mL of saliva in a DNA collection kit (Oragene DNA). DNA extraction from peripheral blood leukocytes was performed by QIAgen while DNA extraction from saliva was performed by DNA genotek. Extracted DNA was sent to the Faculty of Medicine, Chulalongkorn University for next-generation sequencing (NGS). DNA was captured on the TruSeqExome Enrichment Kit (Illumina) and subsequently sequenced on the Hiseq2000 Instrument. The raw data per exome was mapped to the human reference genome hg19 using the Genome browser. Mutation analysis was performed. The DNA of the probands and their parents was Sanger sequenced to confirm the mutation. WES analysis was performed at Macrogen, Inc. (Seoul, Korea) using Variant-Studio™ version 3.0.12 (Illumina, Inc., USA).
CBCT investigation

Six patients received CBCT at the Department of Radiology, Faculty of Dentistry, Chulalongkorn University. 3D images were obtained with Morita’s 3D Accuitomo 170 with 80 kVp, 5mA, exposure time 17.5 seconds, limited field of view of 40x40 mm, and voxel size 0.080 mm. Interpretation and evaluation of scans was performed by a third-year endodontic resident and a 20-year-experienced endodontist. Reliability and interobserver agreement were evaluated by Cohen’s kappa coefficient for categorical variables and demonstrated a kappa value of 1.00, indicating almost perfect agreement. Intraclass correlation coefficients (ICC) were used for evaluation of continuous variables and demonstrated an ICC value of 0.993, indicating excellent agreement. Sectional images of coronal and sagittal views were reviewed with i-Dexel One Volume Viewer software. The coronal plane line was set parallelly to the tooth long axis and perpendicularly to the occlusal surface. One sectional image through the midline of each DE was generated from the sagittal view and another from the coronal view for measurement.

1. Dimensions of DE consist of DE base width and DE cusp height (Fig. 1). The DE base width was the horizontal distance between one side of the base to another side in coronal view. DE cusp height was the vertical distance between the tip and base of DE cusp in coronal view.

2. Main pulp canal extension ratio is calculated by the coronal pulp height divided by the crown height. Crown height was the distance between the buccal cusp tip and cemento-enamel junction. Coronal pulp height was the distance between the pulpal horn and cemento-enamel junction (Fig. 2).

3. Steepness of inclined plane or degree of cuspal inclination measured as the angle between the inclined plane line drawn from buccal or lingual cusp tip to central groove and the imaginary line perpendicularly to the long axis of the teeth (Fig. 3).

![Figure 1](image1.png)  
*Figure 1 Dimension measurement*  
*red line; base width, white line; height*

![Figure 2](image2.png)  
*Figure 2 Main pulp canal extension measurement*  
*red line; crown height, white line; coronal pulp height*

![Figure 3](image3.png)  
*Figure 3 Degree of cuspal inclination*  
*Type I: tubercle, dome-shaped cusp with smooth surface*  
1a) located at inclined plane  
1b) located at central groove  

*Type II: pit, a pinpoint hole that can be detected by explorer no.5, assumed to be the result from a fracture of the DE cusp.*  
2a) located at central groove  

*Type III: cone-like, cone-shaped cusp (flat base with tapering, pointed apex) with base width at least 2x2 mm and apex-base ratio of 0.5 or lesser*  
1a) located at inclined plane  
1b) located at central groove  

This study classified DE according to shape and location (modified from Reichart and Tantiniran, 1975) and pulp extension (modified from Oehlers et al. 1967). Classification of DE shape and location

Type I: cone-like, cone-shaped cusp (flat base with tapering, pointed apex) with base width at least 2x2 mm and apex-base ratio of 0.5 or lesser

1a) located at inclined plane  
1b) located at central groove

Type II: pit, a pinpoint hole that can be detected by explorer no.5, assumed to be the result from a fracture of the DE cusp.

3a) located at inclined plane  
3b) located at central groove
Classification of DE pulp extension (Fig. 4)

For each tooth, sagittal and coronal views of the CBCT images were evaluated and classified as the following:
Class 1: distinct pulp extension to the DEJ at the level of base of DE cusp (occlusal table) in at least 1 radiographic view
Class 2: pulp extension in the dentin (but not reaching DEJ) in at least 1 radiographic view
Class 3: no pulp extension from main pulp canal

If sections from the sagittal and coronal views were indecisive, the axial view at the level of DEJ was evaluated to determine whether pulpal content was present (classified as class 1) or absent (classified as class 2).

Treatment

Teeth diagnosed with pulp necrosis received endodontic treatment while teeth with normal pulp received prophylaxis treatment. 2% Mepivacaine with epinephrine 1:100,000 1.0mL was injected. Rubber dam was applied. The DE cusp was removed by dental burs, followed by preparation of a class I 1.5mm-depth cavity. If the pulpal exposure was marked, the dental pulp was capped with Dycal, subbased with glass ionomer cement (GIC) and restored with resin composite. If the pulp was not exposed, the cavity was lined with GIC and restored with resin composite. Occlusion was checked.

Statistical analysis

Factors including main pulp canal extension, steepness of DE-located inclined plane, size, location and shape of DE and classification of the DE pulpal extension were evaluated. Quantitative variables (pulpal extension, steepness of inclined plane, size) were converted to dichotomous variables by splitting the sample at their means. Associations between each factor and pulpal exposure were evaluated by the Pearson chi-square test or the Fisher’s Exact Test. The level of statistical significance was prespecified at $P < 0.05$. Statistical analyses were performed by SPSS Version 22 software.

Results

Genetic analysis

Four probands (two males and two females) were confirmed with DE by clinical examination. The anomaly was observed in both mother and daughter of two families. Mutation analysis and whole exome sequencing was performed. All variants passed the quality filters including (1) quality score of at least 20 (2) read depth of at least 10 (3) location in the coding regions (4) less than 1% minor allele frequency in the database of 1,000 Genomes Project Consortium, SNPs, Exome Variant Server, and our in-house database of 1,800 Thai exomes. We were able to narrow down the genes of genetic interest to 18 genes (ABCA5, ADAT3, BCORL1, DDR2, ERCC2, HTT, KAT6A, KMT2A, KMT2C, LGI4, MIA3, PLEC, PTCH1, RAI1, TGIF1, TRIP11, WRAP53 and ZIC2). However, a definite pathogenic variant was not identified in all the participants.

CBCT analysis

10 teeth with DE from six participants (four participants had two teeth with DE and two participants had one tooth with DE) were analyzed. There were two teeth classified as type 1 (clinical appearance of type 1 DE tooth shown in Fig. 5A), six teeth classified as type 2
(clinical appearance of type 2 DE tooth shown in Fig. 6A, 7A) and two teeth classified as type 3 (clinical appearance of type 3 DE tooth shown in Fig. 8A). For DE pulp extension, there were two teeth classified as class 1 (3D images shown in Fig. 5C-D), four teeth classified as class 2 (3D images shown in Fig. 7C, 7D) and four teeth with class 3 (3D images shown in Fig. 6C-D, 8C-D). Patient tooth number, sex, age, location and shape of DE, main pulp canal extension, and measurement values were shown in Appendix Tables 1 and 2. Mean values of continuous variables are 0.45 for main pulp canal extension ratio, 33° for degree of buccal cusp inclination (steepness), 26° for degree of lingual cuspal inclination, and 2.2 for size of DE cusp base width. Quantitative numbers were converted to dichotomous variables and listed in the first column of Table 1. Data of factors tested for association with pulpal exposure and statistical results are shown in Table 1. There was a significantly higher chance of pulpal exposure in a DE tooth with a ratio of less than 0.45 and the degree of buccal cusp inclination of less than 33°. Size, shape and location of DE cusp are not statistically related to pulpal exposure.

**Figure 5** Clinical appearance of patient no. 5, tooth 15: Type 1a
A – before treatment, B – after cavity preparation (Arrow indicates pulpal exposure) CBCT section, C – sagittal view, D – coronal view. Class 1

**Figure 6** Clinical appearance of patient no. 3, tooth 34: Type 2b
A – before treatment, B – after cavity preparation CBCT section, C – sagittal view, D – coronal view. Class 3

**Figure 7** Clinical appearance of patient no. 3, tooth 35: Type 2b
A – before treatment, B – after cavity preparation CBCT section, C – sagittal view, D – coronal view. Class 2

**Figure 8** Clinical appearance of patient no. 4, tooth 45: Type 3a
A – before treatment, CBCT section, B – sagittal view, C – coronal view. Class 3
Table 1  Factors tested for association with pulpal exposure

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<sup>a</sup>P<0.05
<sup>b</sup>Fisher’s exact test
<sup>c</sup>Pearson chi-square

Discussions

Genetic inheritance

Previous literature had reported DE in many members of the same family, suggesting possible genetic inheritance of the anomaly.<sup>6-8</sup> In the present study, the anomaly was observed in both the mother and the daughter of two families. Blood or saliva samples taken from two generations in each family were subjected for exome sequencing. Genetic analysis was performed according to the gene list associated with abnormality of the dentition (HP:0000164) The genes of genetic interest were able to be narrowed down to 18 genes. However, a definite pathogenic variant was not identified. Of note, the limitations of this study are 1) There is a limited number of participants with definite phenotypes of DE as the DE is usually worn down before the participants are seen by a dentist., 2). There is a minimal number of affected family members that are available for genetic tests., 3) The causative variant of DE has never been reported, leaving the genetic identification difficult. The causative gene of DE could be a new gene and to determine a new gene as the cause of disease requires many affected and unaffected people within the same family. This would
enable genotype-phenotype segregation to be efficient.
4) Exome sequencing used in this study obtains the data only from the exons coding protein. There is a possibility that the causative variant could lie in the non-coding region of the gene or it can be large gene deletion that requires whole genome sequencing or long-read sequencing, respectively. Based on the above arguments, more samples with definite tooth phenotypes are required that would lead to more possibility to determine the genetic cause of DE. In addition, it was observed that one participant in this study had developmental anomalies comprising supernumerary teeth and brachydactyly. Again, the pathogenic variant was not identified based on the above stated reasons.

CBCT evaluation

Though DE can be easily detected by clinical examination, pulp extension into a DE cusp is not easily gauged. Periapical radiographs had been used in previous literature to confirm pulp horn extension.\(^9,10\) However, the anomaly is difficult to examine by intraoral radiograph due to overlapping of the lingual cusps. The extension of the pulp might be inconclusive. CBCT enables three-dimensional investigation of DE and its pulpal content. The data gained from evaluating CBCT sections can be used to identify correlation between pulpal extension and clinical pulpal exposure and confirm the preciseness of periapical radiographs for DE evaluation.

Previous literature had reported cases with pulpal infection resulting from traumatic occlusion and fracture of DE.\(^6,11-14,18\) To prevent apical periodontitis, prophylaxis treatment by removal of tubercle followed by cavity preparation and filling with resin composite under rubber dam application was suggested.\(^14\) Pulpal depth of 1.5 mm from the central groove was required to resist occlusal forces and prevent composite filling from fracture.\(^20\)

The present study examined radiographic morphology of DE teeth and assessed correlations between CBCT findings and clinical pulpal exposure. A field of view of 40x40 mm and the smallest voxel size of 0.080 mm were selected to obtain good quality 3D images for pulp extension evaluation. In this study, the ratio between coronal pulp height and crown height was used to evaluate the main pulp extension of each tooth. From the results, if the coronal pulp height extends above half of the crown height (ratio > 0.5), there is a high chance of pulpal exposure after tubercle fracture or after prophylaxis cavity preparation. Main pulp extension is related to the degree of development of the tooth since during maturation secondary dentin is continuously deposited causing narrowing of the dental pulp cavity. The age of the patient at the time of tubercle fracture or receiving prophylaxis cavity preparation is one of the factors influencing pulpal exposure. Therefore, if DE is detected by the dentist while the main pulp canal extension ratio is greater than 0.5, other prophylaxis treatment such as grinding of the tubercle to encourage the deposition of secondary dentin or resin sealant over the tubercle could be alternative treatment options to avoid pulpal exposure.

Steepness of buccal cusp is also associated with pulpal exposure, especially when the tubercle is located at an inclined plane. The degree of cuspal inclination represents the steepness of an inclined plane. The results of this study show that pulpal exposure occurs in the teeth that have the degree of buccal cuspal inclination at 33° or less. The higher the degree, the lower risk of pulpal exposure is due to the longer distance between pulp horn and external tooth surface.

DE located at the buccal inclined plane of the lingual cusp is reported as a variation.\(^2,7\) In this study, DE cusp of all recruited participants were found at the lingual inclined plane of the buccal cusp or at the central groove. Hence, the statistical relationship between lingual cusp steepness and clinical pulp exposure was not established.

Fracture of tubercle, which leads to pulpal exposure, is related with interfered occlusion of DE cusp with the opposing tooth upon the cusp-like elevation.\(^2,9,10,12\)
On the other hand, location of the tubercle is unlikely related to pulpal exposure. Alignment and axis of the tooth define whether the tubercle is in contact with the opposing tooth, causing traumatic occlusion during function followed by fracture or wear of the tubercle or not. In one patient who had DE of teeth 35 (mandibular left second premolar) and 45 (mandibular right second premolar), DE were observed at the same location and both appeared in the form of a circular-based flat surface. Tooth 45 had a deep pit and was diagnosed with pulp necrosis while tooth 35 had a smooth occlusal surface with a dentinal core. From radiographic findings, tooth 45 had an immature root apex with wide open apical foramen. Therefore, pulp necrosis might have occurred while the root was incompletely formed. It can be assumed that the cause of the pulpal disease for tooth 45 was due to a sudden fracture of the tubercle, forming a deep pit that enhanced bacterial accumulation followed by tooth decalcification and pulpal inflammation. On the contrary, tooth 35 had normal pulp tissue with mature root apex. Tubercle was assumed to be grinded and worn, forming a flat surface with dentin deposition.

The relationship between the original shape of DE and pulpal exposure has not been reported in previous literature. From the results, the original shape is not directly associated with pulpal exposure. However, if the tubercle is fractured and DE appears in the form of a pit, the tooth is more prone to caries leading to pulpal disease since the pit can enhance bacterial accumulation and tooth decalcification.

In another patient with DE of teeth 14 (maxillary right first premolar) and 15 (maxillary right second premolar), after receiving cavity preparation for prophylaxis treatment, tooth 14 showed smooth dentinal core while tooth 15 showed pinpoint pulpal exposure. From radiographic evaluation, the main pulp canal extension ratio of tooth 14 was lower than that of tooth 15 (0.44 and 0.52 respectively), and the degree of the buccal cusp inclination of tooth 14 is higher than tooth 15 (49° and 30° respectively). In addition, in the patient with DE of teeth 34 (mandibular left first premolar) and 35 (mandibular left second premolar), tooth 34 with normal pulp had a lower main pulp canal extension ratio than tooth 35 with necrotic pulp (0.43 and 0.57 respectively). Furthermore, the degree of the buccal cusp inclination of tooth 34 was higher than tooth 35 (32° and 20° respectively). These results suggested that main pulp canal extension ratio and degree of buccal cusp inclination are influencing factors for pulpal exposure of a fractured DE.

The examination of DE using CBCT in this study enables three-dimensional investigation of the radiographic appearance of DE and its pulpal content. The ratio of main pulp canal extension has been proven to be associated with clinical pulpal exposure after DE fracture or prophylaxis cavity preparation. The CBCT in clinical practice is recommended for treatment of dens evaginatus using regenerative endodontics procedures. Periapical radiographic films can be used to estimate the main pulp canal extension, assisting clinicians to determine the best treatment for each patient.

Factors influencing clinical pulpal exposure in the teeth with DE are the main pulp canal extension and DE-located cusp steepness. Radiographs can be used to estimate chances of pulp exposure if prophylaxis cavity preparation is required. More samples with definite tooth phenotypes of DE are required to precisely determine the causative genetic variants of DE.

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Conclusion

Factors influencing clinical pulpal exposure in the teeth with DE are the main pulp canal extension and DE-located cusp steepness. Radiographs can be used to estimate chances of pulp exposure if prophylaxis cavity preparation is required. More samples with definite tooth phenotypes of DE are required to precisely determine the causative genetic variants of DE.

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