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INVESTIGATION OF AMELX rs17878486 AND DSPP c.49C>T GENETIC POLYMORPHISMS AS POTENTIAL MARKERS OF THE DEVELOPMENT OF ENAMEL HYPOPLASIA IN CHILDREN

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Enamel hypoplasia is a multifactorial disorder of amelogenesis associated with genetic variants.

The aim of the study was to evaluate the association of AMELX rs17878486 and DSPP c.49C>T with enamel hypoplasia in children.

Materials and Methods. Twenty-three children aged 6–8 years were examined: 14 with enamel hypoplasia and 9 without enamel hypoplasia. Genotyping was performed using real-time PCR.

Results. The C allele of AMELX rs17878486 was more frequent in children with enamel hypoplasia; however, the association was not statistically significant. No statistically significant association between DSPP c.49C>T and enamel hypoplasia was found.

Conclusions. For AMELX rs17878486, a trend toward a higher frequency of the allele C was observed in children with enamel hypoplasia, which requires confirmation in a larger sample; for DSPP c.49C>T, no statistically significant association was found.

Keywords: enamel hypoplasia, AMELX rs17878486, DSPP c.49C>T, genetic polymorphisms, children.

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ДОСЛІДЖЕННЯ ГЕНЕТИЧНИХ ПОЛІМОРФІЗМІВ AMELX rs17878486 ТА DSPP c.49C>T ЯК МОЖЛИВИХ МАРКЕРІВ РОЗВИТКУ ГІПОПЛАЗІЇ ЕМАЛІ У ДІТЕЙ

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Гіпоплазія емалі є мультифакторним порушенням амелогенезу, пов'язаним із генетичними варіаціями. Мета дослідження – оцінити асоціацію AMELX rs17878486 та DSPP c.49C>T з гіпоплазією емалі у дітей.

Обстежено 23 дитини віком 6–8 років: 14 з гіпоплазією та 9 без патології. Генотипування проводили методом ПЛР у реальному часі. У разі гіпоплазії частіше виявляли алель С AMELX rs17878486, проте асоціація не була статистично значущою. Для DSPP c.49C>T статистично значущої асоціації з гіпоплазією не встановлено. Для AMELX rs17878486 виявлено тенденцію до більшої частоти алеля С у дітей з гіпоплазією емалі, яка потребує підтвердження на більшій вибірці; для DSPP c.49C>T статистично значущої асоціації не встановлено.

Ключові слова: гіпоплазія, генетичні поліморфізми AMELX rs17878486 та DSPP c.49C>T, діти.

Introduction

Enamel hypoplasia is a disorder of enamel formation. It develops during the period of active secretion of the enamel matrix by ameloblasts. The etiology of enamel hypoplasia is multifactorial and includes genetic, systemic, and exogenous factors. Systemic causes include calcium, phosphorus, and vitamin D deficiency, intrauterine pathology, preterm birth, low birth weight, as well as infectious diseases accompanied by fever during the period of tooth formation [1; 2]. Toxic exposures, including excessive fluoride intake, as well as local injury or inflammation in the area of the tooth germ, also play an additional role [1; 3].

A separate and fundamentally important group of causes comprises genetically determined disorders of amelogenesis. Congenital defects in genes that regulate ameloblast function and the synthesis of enamel matrix proteins lead to hypoplasia even in the absence of concomitant factors [4; 5]. It is at this level that the link between genetic variations and morphological enamel defects is established.

The AMELX gene encodes amelogenin, the major structural protein of the organic enamel matrix, which plays a key role in regulating the growth, orientation, and aggregation of hydroxyapatite crystals [5–7]. Impaired

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expression or structure of amelogenin leads to defects in the secretory stage of amelogenesis, directly resulting in a reduced amount of enamel formed [5; 6]. AMELX mutations are associated in a substantial proportion of cases with a hypoplastic or combined hypoplastic-hypomineralized phenotype [7; 8]. The T allele and TT genotype of rs17878486 are associated with developmental defects of the enamel of primary teeth [9]. AMELX rs17878486 affects not only enamel formation but also its resistance to demineralization [10]. Recent genotype-phenotype studies have shown that both coding and intronic AMELX variants can disrupt splicing and mRNA stability, resulting in amelogenin deficiency and the formation of thin, fragmented, or unevenly formed enamel [8]. Thus, AMELX is one of the key genes involved in enamel formation, and its variants are considered among the major genetic determinants of enamel hypoplasia in humans [5; 6; 11].

The DSPP gene encodes dentin sialophosphoprotein, which is a precursor of two major non-collagenous dentin proteins, dentin sialoprotein (DSP) and dentin phosphoprotein (DPP), which determine the mineralization, organization, and mechanical properties of dentin [12; 13]. Pathogenic DSPP variants are the main cause of dentin dysplasia and are associated with structural instability of the tooth crown [12–14]. Although DSPP is predominantly expressed in odontoblasts, studies have demonstrated its involvement in the formation of the dentin-enamel junction [15]. Enamel defects, including areas of hypoplasia and abnormalities of the superficial enamel structure, have been described in carriers of DSPP mutations, suggesting a possible indirect effect of this gene on enamel formation through altered dentin support and tissue interactions [15]. The role of DSPP in the development of enamel hypoplasia is considered to be modifying and is manifested predominantly within disorders of the enamel-dentin complex [12; 15].

The selection of the single-nucleotide polymorphisms AMELX rs17878486 (T>C) and DSPP c.49C>T for this study was based on evidence of their association with disorders of tooth hard tissue formation.

Thus, AMELX rs17878486 and DSPP c.49C>T were selected as candidate single-nucleotide polymorphisms to assess their potential role as genetic markers of the risk of enamel hypoplasia in children.

Aim of the study – to evaluate the association of the single-nucleotide polymorphisms AMELX rs17878486 and DSPP c.49C>T with enamel hypoplasia in children.

Materials and Methods

The study was conducted as a pilot study. A total of 23 children aged 6–8 years were included in the study and divided into two groups: a normal group and a pathology group. The pathology group included 14 children with delayed tooth eruption and clinically confirmed enamel hypoplasia of varying severity. The normal group included 9 children without signs of impaired enamel formation. The condition of the hard tissues of the teeth was assessed during a clinical dental examination. Developmental enamel defects were recorded using the DDE (Developmental Defects of Enamel) index according to the FDI criteria. The permanent first molars and incisors were assessed. Enamel hypoplasia was diagnosed based on the presence of quantitative enamel defects and differentiated from molar-incisor hypomineralization (MIH) and fluorosis.

The study was approved by the Commission on Ethical Issues and Biomedical Ethics of Odesa National Medical University (Protocol No. 10 dated November 18, 2019) and was conducted with the written consent of the participants and in accordance with the bioethical principles set forth in the Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" and the "Universal Declaration on Bioethics and Human Rights" (UNESCO).

Buccal epithelial samples were collected using sterile swabs. Genomic DNA was extracted using a modified method with Chelex. The concentration of the obtained DNA was 50 ng/μL. Polymerase chain reaction was performed in a total volume of 20 μL, with 2.5 μL of DNA-containing supernatant added.

Genotyping of the AMELX rs17878486 and DSPP c.49C>T polymorphisms was performed by PCR with real-time detection of amplification products and melting curve analysis. Amplification was performed using a DT-Lite real-time PCR detection amplifier (DNA-Technology LLC). Fluorescence signal acquisition and interpretation of the melting curves were performed automatically using the instrument's built-in software.

The conformity of genotype distributions to Hardy–Weinberg equilibrium was assessed using the χ^2 test. Fisher's exact test was used to compare genotype and allele frequencies between the groups. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed in the R environment (version 4.5.1) using the epitools package.

Research results and their discussion

Table 1 presents the distribution of genotypes of the AMELX rs17878486 and DSPP c.49C>T polymorphisms in the normal and pathology groups. For the AMELX rs17878486 polymorphism, the frequency of the C allele was 53.6 % in the enamel hypoplasia group and 27.8 % in the normal group. For DSPP c.49C>T, the frequency of the T allele was 25.0 % and 11.1 %, respectively.

Table 2 presents the results of the analysis of the association between the AMELX rs17878486 polymorphism and enamel hypoplasia under different genetic models. No statistically significant association between AMELX rs17878486 and the presence of enamel hypoplasia was identified in any of the analyzed models. In most models, the OR values for the C allele were greater than 1; however, the 95 % confidence intervals were wide and included 1.

Table 3 presents the results of the analysis of the association between the DSPP c.49C>T polymorphism and enamel hypoplasia under different genetic models. For the DSPP c.49C>T polymorphism, no statistically significant association with enamel hypoplasia was identified under any of the analyzed inheritance models ($p>0.05$). The wide confidence intervals indicate substantial uncertainty in the estimated effects. Overall, the DSPP c.49C>T polymorphism was not statistically significantly associated with enamel hypoplasia in the study sample.

Table 1

Frequencies of genotype distribution in different groups of study participants

Gene	Polymorphic variants	Normal N	Normal %	Hypoplasia N	Hypoplasia %
AMELX: T>C [rs17878486]	TT TC CC	4 5 0	44.4 55.6 0	3 7 4	21.4 50 28.6
DSPP: C>T [c.49]	CC TC TT	7 2 0	77.8 22.2 0	8 5 1	57.2 35.7 7.1

Table 2

Distribution of polymorphic variants of the AMELX: T>C [rs17878486] gene

Model	Polymorphic variants	Statistical analysis results			
		c	OR	95 % CI	p
Additive model	TT TC CC	3.161	0.341	0.055–2.131	0.075
			0.800	0.149–4.297	
			8.143	0.387–172.070	
Recessive model	CC TT+TC	1.442	8.143	0.387–172.070	0.230
			0.123	0.006–2.595	
Dominant model	TT TC+CC	0.449	0.341	0.055–2.131	0.480
			2.933	0.469–18.334	
Overdominant model	TT+CC TC	0.000	1.250	0.233–6.715	1.000
			0.800	0.149–4.297	
Multiplicative model	T C	2.010	0.333	0.094–1.188	0.156
			3.000	0.842–10.693	

Note: c – logarithm of the odds ratio (logOR); OR – odds ratio for the respective genotypes; 95 % CI – 95 % confidence interval; p – statistical significance level.

Table 3

Distribution of polymorphic variants of the DSPP c.49C>T gene

Model	Polymorphic variants	Statistical analysis results			
		c	OR	95 % CI	p
Additive model	CC CT TT	1.300	0.381	0.057–2.534	0.254
			1.944	0.287–13.188	
			2.111	0.077–57.613	
Recessive model	TT CC+CT	0.000	2.111	0.077–57.613	1.000
			0.474	0.395–17.459	
Dominant model	CC CT+TT	0.320	0.381	0.057–2.534	0.572
			2.625	0.395–17.459	
Overdominant model	CC+TT CT	0.049	0.514	0.076–3.488	0.824
			1.944	0.287–13.188	
Multiplicative model	C T	0.605	0.375	0.068–2.054	0.437
			2.667	0.487–14.609	

Note: Abbreviations are as defined in Table 2.

In this study, the associations of the AMELX rs17878486 and DSPP c.49C>T polymorphisms with enamel hypoplasia in children were analyzed. For AMELX rs17878486, a higher frequency of the C allele was observed in children with enamel hypoplasia compared with the normal group. None of the genetic models examined demonstrated a statistically significant association. The wide confidence intervals indicate substantial uncertainty in the estimates, which may be attributable to the limited sample size. Given the pilot nature of the study and the absence of statistically significant associations, the findings should be considered preliminary.

For the DSPP c.49C>T polymorphism, no statistically significant association with enamel hypoplasia was established. The wide confidence intervals do not allow a definitive conclusion to be drawn regarding the possible effect of this variant in the study sample.

Overall, the findings are consistent with the current concept that enamel hypoplasia results from disturbances in genes that directly control the enamel matrix, primarily AMELX, whereas dentin-related genes, including DSPP, may affect the phenotype indirectly through the condition of the dentin-enamel complex. This highlights the need for further studies involving larger samples and a broader range of single-nucleotide polymorphisms.

The main limitation of the study is the small sample size, which reduces the statistical power of

the analysis and results in wide confidence intervals for the estimated effects. Therefore, the findings are preliminary and require confirmation in larger independent samples.

Conclusions

1. In children with enamel hypoplasia, a tendency toward a higher frequency of the C allele of the AMELX rs17878486 polymorphism was observed compared with the normal group; however, no statistically significant association was established.

2. No statistically significant association between the DSPP c.49C>T polymorphism and enamel hypoplasia was established in the study sample.

3. The limited sample size reduces the statistical power of the analysis, necessitating further studies in larger cohorts to clarify the possible role of the studied polymorphisms in the development of enamel hypoplasia.

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