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PREDICTION TO HYPERTROPHIC FORMS OF GINGIVITIS IN CHILDREN WITH GENETIC ASSESSMENT

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The study is dedicated to searching for genetic factors that determine the predisposition to children's hypertrophic and catarrhal forms of gingivitis. The study involved children with hypertrophic gingivitis (12 patients) and chronic catarrhal gingivitis (16 patients). Because of the study, it was found that hypertrophic gingivitis and chronic catarrhal gingivitis differed significantly in the distribution of polymorphism genotypes TNFRSF1B, MMP1B and TGFB1 in examined patients. No difference was found between the groups by rs1800629 TNF-alpha-308G>A, rs1800795 IL6-174G>C, rs3024491 IL10-1082G>A and rs1800012 COL1A1 IVS1 2046G>T polymorphisms. The results obtained, in our opinion, should be taken into account in the development of treatment and prevention measures to support the dental treatment of children with hypertrophic gingivitis.

Key words: children, periodontal tissue diseases, gene polymorphisms, buccal epithelium, polymerase chain reaction.

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ГЕНЕТИЧНА ОЦІНКА СХИЛЬНОСТІ ДО ГІПЕРТРОФІЧНОЇ ФОРМИ ГІНГІВІТУ У ДІТЕЙ

Дослідження присвячено пошуку генетичних чинників, що визначають схильність до гіпертрофічної та катаральної форм гінгівіту у дітей. В дослідженні приймали участь групи дітей з гіпертрофічним гінгівітом (12 пацієнтів) та з хронічним катаральним гінгівітом (16 пацієнтів). У результатах проведеного дослідження було виявлено, що гіпертрофічний гінгівіт і хронічний катаральний гінгівіт суттєво відрізнялися за розподілом генотипів поліморфізму TNFRSF1B, MMP1B і TGFB1 у обстежених пацієнтів. Не було знайдено будь-якої різниці між групами за поліморфізмами rs1800629 TNF-alpha-308G>A, rs1800795 IL6-174G>C, rs3024491 IL10-1082G>A та rs1800012 COL1A1 IVS1 2046G>T. Отримані результати, на наш погляд, необхідно враховувати при розробці лікувально-профілактичних заходів супроводження стоматологічного лікування дітей з гіпертрофічним гінгівітом.

Ключові слова: діти, захворювання тканини пародонту, поліморфізми генів, букальний епітелій, полімеразна ланцюгова реакція.

The study is a fragment of the research project "Correction of pathogenetic mechanisms of carbohydrate and lipid metabolism disorders in the body and tissues of the oral cavity in patients depending on environmental and nutritional factors affecting carbohydrate and lipid metabolism", state registration No. 0118U006966.

Hypertrophic gingivitis (HG) is accompanied by hyperplastic processes in the form of reactive growth of fibrous elements of the connective tissue base and basal cells of the gum epithelium. The immediate causes of hypertrophic gingivitis can be local factors such as occlusal and individual tooth abnormalities, unsatisfactory prosthetic constructions, improper fillings, dental plaque, poor hygiene at wearing orthodontic appliances and common factors: diseases of the nervous system, endocrine system leukemic reticulosis), medication, vitamin C deficiency, hormonal changes in the body [15].

The nature of the inflammatory reaction to plaque bacteria and hyperplastic processes in the tissues of the gums are deeply individual and largely determined by genetic factors [7]. A number of studies have shown that carriers of certain alleles of some cytokine genes have an increased risk of gingivitis or periodontitis [14].

TGF-β1 is a multifunctional cytokine involved in the epithelial-mesenchymal transition, in which epithelial cells acquire the phenotypic properties of mesenchymal cells, which leads to fibrosis of various organs [10]. The rs1800471 TGFB1 915 G>C (Arg25Pro) polymorphism in codon 25 is associated with the replacement of arginine by proline.

MMP1, or intratissue collagenase, is a member of the matrix metalloprotease family, extracellular zinc-dependent endopeptidases that play an important role in the remodeling and destruction of all types of extracellular matrix proteins. MMP together with the tissue inhibitor of metalloproteinases TIMP1 play an important role in the physiological remodeling of the periodontium and the response to mechanical action during orthodontic treatment. Inhibition of MMP by synthetic inhibitors has been shown to reduce orthodontic movement of teeth. Rs1799750 is a relatively common insertional polymorphism at position-1607 of the MMP1 gene promoter. It is believed that the insertion genotype (2G/2G) leads to higher transcriptional activity of this gene, which contributes to more intense collagen breakdown [9].

Thus, the identification and verification of genetic markers of periodontal disease and the introduction of biomarkers in clinical practice should improve the diagnosis of periodontal disease, monitor their progression and response to treatment, as well as the development of personalized medicine in periodontology.

The purpose of the study was to establish the genetic factors that determine the predisposition to hypertrophic and catarrhal forms of gingivitis in children.

Materials and methods. The group of children with hypertrophic gingivitis (HG) included 12 children (8 patients with grade 1–2 fibrous form and 4 patients with grade 1 edematous form). The group of patients with chronic catarrhal gingivitis (CCG) included 16 children (12 patients with local CCG grade 1–2 and 4 patients with severe CCG grade 2–3).

Isolation of DNA from buccal epithelial cells was performed according to a modified method using Chelex 200μl of a 5 % solution of Chelex [12]. 200 μl of a 5 % solution of Chelex 100 in sterile distilled water (Chelex sodium, 100–200 mesh, Bio-Rad) was added to a test tube (Eppendorf) containing an epithelial cell scraping applicator. Before adding the resin, it was stirred to a homogeneous state with a pipette with a wide hole and an aliquot was taken directly during stirring. Incubate at 56° C for 30 min with constant stirring on a thermoshaker. Then incubated at 96° C for 8 min, requires shaking periodically. After incubation, it is centrifuged for 3 min at 12,000 g (Eppendorf Centrifuge 5424). The concentration and purity of the DNA specimen were determined spectrophotometrically (Nanophotometr, Implen), taking an aliquot of 5 μl directly from the tube with the DNA solution. For polymerase chain reaction (PCR), 5 μl of supernatant was selected.

Allelic variants of rs1800629 polymorphisms TNF-alpha-308G>A, rs1800795 IL6-174G>C, rs3024491 IL10-1082G>A, rs1799750 MMP1-1607 ins G, rs1800471 TGFB1 915 G>C evaluated by allele-specific polymerase chain reaction (PCR) [4, 5]. Amplification of the studied gene regions was performed in parallel in two tubes (Eppendorf) for the normal and mutant alleles of each gene in 20 μl of buffer solution and 100 nm of each oligonucleotide primer using kits "SNP-express-EF" NPF Litech-150 NG DNA. The control sample was diluted with 5 μl of diluent in both types of reaction mixture and amplified on an Analytik Jena thermal cycler (Flex Cycler, Germany) according to the manufacturer's instructions.

Polymorphisms rs1800012 COL1A1 IVS1 2046G>T and rs590368 TNFRSF1B-3609C>T were genotyped by PCR-RFLP (restriction fragment length polymorphism) with appropriate primers. Amplification was performed on a thermal cycler "Analytik Jena" (Flex Cycler, Germany). To detect allelic variants of rs1800012 COL1A1 IVS1 2046G>T amplicons were treated with restriction enzyme PvuII (Fermentas, Lithuania), rs590368 TNFRSF1B-3609C>T – restriction enzyme M3IF.

Fractionation of the amplification and restriction products was performed in a horizontal 2 % agarose gel prepared on disposable trisborate buffer (1xTBE) at a voltage of 100V for 45 minutes. Molecular weight marker – pUC19 DNA: MSP1. Agarose gel was stained with ethidium bromide and visualized in ultraviolet light.

Statistical analysis of the results, including the Hardy-Weinberg Equilibrium (HWE) test and the assessment of the association of genotypes and alleles with CCG and hypertrophic gingivitis by Pearson's χ^2 method, was performed using the DeFinetti genetic statistics program. Associations were characterized by an odds ratio (OR) with a 95 % confidence interval. Values of $p<0.05$ were considered statistically significant.

Results of the study and their discussion. Genotyping of groups of children with HG (n=12) and CCG (n=16) by single nucleotide polymorphisms rs1800629 TNF-alpha-308G>A, rs1800795 IL6-174G>C, rs560F9-1082G>A, rs1800012 COL1A1 IVS1 2046G>T, rs1799750 MMP1-1607 ins G and rs1800471 TGFB1 915 G>C (Arg25Pro) [2]. The frequency distribution of genotypes, the correspondence of their distribution to Hardy-Weinberg Equilibrium (HWE), as well as the differences between the groups regarding the frequency distribution of genotypes and alleles were analyzed in the studied groups. Deviations in the distribution of genotypes from those theoretically calculated in HWE were found in the group of hypertrophic gingivitis for rs590368 TNFRSF1B-3609C>T, in the group with CCG for rs1800012 COL1A1 IVS1 2046G>T and for rs1800471 TGFB1 915 G>C (Arg25Pro) in both groups.

Distribution and comparison of frequencies of genotypes and alleles of polymorphisms of TNFα, TNFRSF1B, COL1A1 and MMP1 genes in different groups of children with chronic catarrhal gingivitis and hypertrophic gingivitis are presented in tables 1 and 2.

Table 1

Distribution and comparison of frequencies of genotypes and alleles of polymorphisms of TNFα, TNFRSF1B genes in examined children

Poly-morphism	rs1800629 TNF-alpha-308G>A					
Genotype, allele	GG	GA	AA	G	A	HWE p-value
CCG, n (frequency)	6 (0.375)	10 (0.625)	0 (0.000)	22 (0.688)	10 (0.312)	0.199
HG, n (frequency)	4 (0.333)	8 (0.667)	0 (0.000)	16 (0.667)	8 (0.333)	0.148
Frequency comparison	A<>G	GA<> GG	GA+AA<>GG *DM*	AA<>GG+GA *RM*	–	–
OR (95 % CI)	1.100 (0.222–5.445)	1.200 (0.130–11.052)	1.200 (0.130–11.052)	1.400 (0.020–97.428)	–	–
χ^2 p-value	1.001	0.872	0.872	1.000	–	–
rs590368 TNFRSF1B-3609C>T						
Genotype, allele	CC	CT	TT	C	T	HWE p-value
CCG, n (frequency)	10 (0.625)	6 (0.375)	0 (0.000)	26 (0.813)	6 (0.187)	0.514
HG, n (frequency)	4 (0.333)	0 (0.000)	8 (0.667)	8 (0.333)	16 (0.667)	0.014
Frequency comparison	T<>C	CT<> CC	CT+TT<>CC *DM*	TT<>CC+CT *RM*	–	–
OR (95 % CI)	8.667 (1.526–49.220)	0.314 (0.011–8.684)	3.333 (0.362–30.701)	19.800 (0.744–527.260)	–	–
χ^2 p-value	0.019	0.301	0.280	0.022	–	–

N o t e . CI – confidence interval; DM – dominant model; RM – recessive model.

Table 2

Distribution and comparison of frequencies of genotypes and alleles of polymorphisms of COL1A1, MMP1 genes in examined children

Poly-morphism	rs1800012 COL1A1 IVS1 2046G>T					
Genotype, allele	GG	GT	TT	G	T	HWE p-value
CCG, N (frequency)	10 (0.625)	2 (0.125)	4 (0.250)	22 (0.688)	10 (0.312)	0.045
HG, N (frequency)	2 (0.167)	6 (0.500)	4 (0.333)	10 (0.417)	14 (0.583)	0.944
Frequency comparison	T<>G	GT<> GG	GT+TT<>GG *DM*	TT<>GG+GT *RM*	–	–
OR (95 % CI)	3.080 (0.647–14.662)	15.000 (0.663–339.55)	8.333 (0.631–110.022)	5.000 (0.273–91.518)	–	–
χ^2 p-value	0.152	0.065	0.086	0.260	–	–
rs1799750 MMP1-1607 ins G						
Genotype, allele	1G1G	1G2G	2G2G	1G	2G	HWE p-value
CCG, N (frequency)	10 (0.625)	4 (0.250)	2 (0.125)	24 (0.750)	8 (0.250)	0.346
HG, N (frequency)	2 (0.167)	2 (0.167)	8 (0.666)	6 (0.250)	18 (0.750)	0.174
Frequency comparison	2G<> 1G	1G2G<>1G1G	1G2G+2G2G<>1G1G *DM*	2G2G<> 1G1G+1G2G *RM*	–	–
OR (95 % CI)	9.000 (1.598–50.691)	2.500 (0.100–62.605)	8.333 (0.631–110.022)	20.000 (0.930–429.904)	–	–
χ^2 p-value	0.009	0.570	0.086	0.036	–	–

Note. CI – confidence interval; DM – dominant model; RM – recessive model

In the study in the MMP1 gene, the insertion of G, i.e. the 2G allele was associated with HG: OR 9,000 (95 % CI 1,598–50,691), the reliability of the value of χ^2 p=0.009. The incidence of the 2G allele in the hypertrophic gingivitis and chronic catarrhal gingivitis groups was 75 % and 25 %, respectively; frequency of homozygotes 2G2G of the group of hypertrophic gingivitis was 67 % against the 13 % of the group of chronic catarrhal gingivitis. The association of this polymorphism with hypertrophic gingivitis

corresponded to the recessive model of inheritance (2G2G<>1G1G+1G2G): the reliability of the value of χ^2 p=0.036. Thus, the polymorphism rs1799750 MMP1-1607 ins G may be a factor in the predisposition to hypertrophic gingivitis.

Distribution and comparison of genotypes and alleles of polymorphisms of TGFB1 gene in different groups of children with chronic catarrhal gingivitis and hypertrophic gingivitis are presented in fig. 1.

We have identified differences between the groups in distribution of genotypes and alleles of three polymorphisms: rs590368 TNFRSF1B-3609C>T, rs1799750 MMP1-1607 ins G and rs1800471 TGFB1 915 G>C (Arg25Pro). The T-allele of the TNFRSF1B gene was associated with hypertrophic gingivitis: OR 8,667 (95 % CI 1,526–49,220), significance of χ^2 p=0.019. The frequency of T-allele in the groups with hypertrophic gingivitis and chronic catarrhal gingivitis was 67 % and 19 %, respectively, the frequency of homozygotes TT was 67 % in the group with hypertrophic gingivitis against 0 % in the group with chronic catarrhal gingivitis. The TT genotype was associated with the risk of hypertrophic gingivitis: reliability of the value of χ^2 p=0.022, which corresponds to the recessive model (TT<>CC+CT) of the association of polymorphism rs590368 TNFRSF1B-3609C>T with this disease.

Distribution and comparison of genotypes and alleles of polymorphisms of IL6 gene in different groups of children with chronic catarrhal gingivitis and hypertrophic gingivitis are presented in fig. 2.

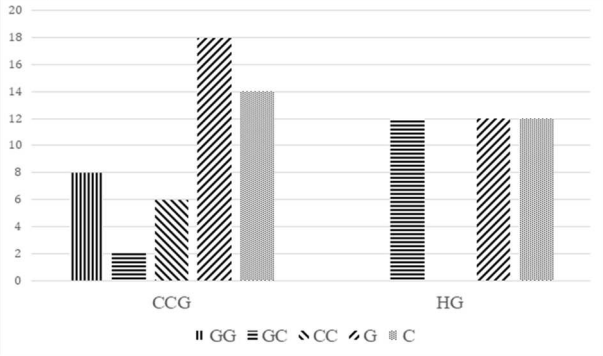

Fig.1. Distribution and comparison of genotypes and alleles of polymorphisms of TGFB1 gene in examined children

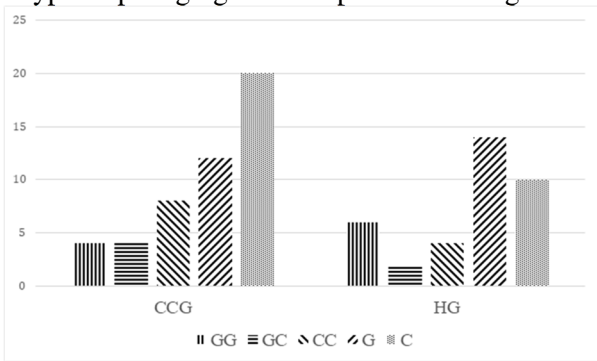

Fig.2. Distribution and comparison of genotypes and alleles of polymorphisms of IL6 gene in examined children

In our study, the group of hypertrophic gingivitis consisted of a genotype of only heterozygotes of GC, while in the group of chronic catarrhal gingivitis the frequency of heterozygotes was 13 %. Thus, the GC genotype was associated with hypertrophic gingivitis: OR 39,000 (95 % CI 1,277–1190,838), which corresponded to the dominant association model (GC+CC<>GG).

Distribution and comparison of genotypes and alleles of polymorphisms of IL6 gene in different groups of children with chronic catarrhal gingivitis and hypertrophic gingivitis are presented in fig. 3.

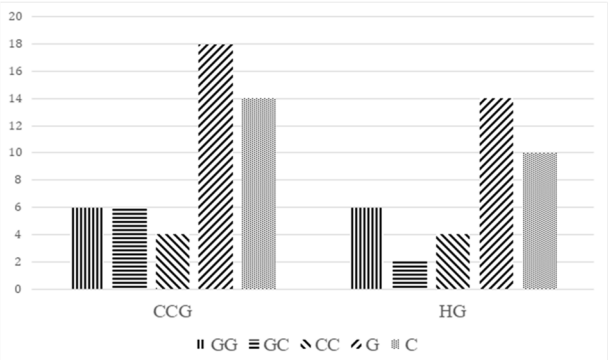

Fig.3. Distribution and comparison of genotypes and alleles of polymorphisms of IL10 gene in examined children

The chart above shows that the rs3024491-1082G>A polymorphism in the anti-inflammatory cytokine gene IL10 was not associated with gingivitis.

It is known that the CC genotype of TNF polymorphism is associated with fewer CD14+cells encoding TNFRII compared to the CT genotype [13]. Based on these data, we can assume that the TT genotype is associated with increased TNFR2 activity and decreased apoptosis, which is one of the prerequisites for the growth of fibrous tissue in hypertrophic gingivitis [3]. The TNFRSF1B−3609C>T polymorphism may be a candidate for the genetic determinants of hypertrophic gingivitis, especially its fibrous form. The G-allele of the rs1800795 IL6-174G>C polymorphism is associated with increased expression of the proinflammatory cytokine interleukin 6. This polymorphism is also associated with the risk of periodontal disease, including gingivitis [11]. The rs3024491-1082G>A polymorphism in the anti-inflammatory cytokine gene IL10 was also not associated with gingivitis in a study by Scapoli L [11]. In our study, however, we failed to differentiate between different forms of gingivitis based on the genotype of the rs1800795 polymorphism. TNFRSF1B encodes a TNFR2 membrane protein that belongs to the TNFα tumor necrosis factor receptor superfamily along with TNFR1. If the binding of TNFα to TNFR1 induces

cellular apoptosis, TNFR2 activation is associated with cell proliferation and survival. TNFR2 expression is associated with the proliferation of various tumors [15]. In different populations, the frequency of the TT genotype is 35–168 %. Hypertrophic gingivitis group in our study demonstrated a significant advantage of this genotype compared to chronic catarrhal gingivitis and the average prevalence in populations. It should be noted that in [6] the GC genotype was more common in the case of gastric cancer against controls. At the same time, data on the association of a particular allele with the level of TGF-β1 in serum are very contradictory [1, 8], which requires further research. In our study, the groups chronic catarrhal gingivitis and hypertrophic gingivitis did not differ significantly in the frequency distribution of genotypes and alleles of polymorphisms rs1800629 TNF-alpha-308G>A, rs1800795 IL6-174G>C, rs3024491 IL10. Analyzing the obtained information, it can be concluded that the acquired results, in our opinion, should be taken into account in the development of treatment and prevention measures to support the dental treatment of children with hypertrophic gingivitis.

Conclusions

1. Hypertrophic gingivitis and chronic catarrhal gingivitis differed significantly in the distribution of polymorphism genotypes TNFRSF1B, MMP1B and TGFB1 in examined patients.

2. We did not find any difference between the groups of rs1800629 TNF-alpha-308G>A, rs1800795 IL6-174G>C, rs3024491 IL10-1082G>A and rs1800012 COL1A1 IVS1 2046G>T polymorphisms.

3. The results obtained, in our opinion, should be taken into account in the development of treatment and prevention measures to support the dental treatment of children with hypertrophic gingivitis.

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