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DEFB1, MMP9 AND COX2 GENE POLYMORPHISMS AND PERIODONTITIS

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The study is devoted to finding the association of single-nucleotide polymorphisms rs1799946–52G>A of the DEFB1 gene, rs2274756–8202 A>G of the MMP9 gene and rs689466–1195 A>G of the COX2 gene with periodontitis in the Ukrainian population. 34 patients participated in the research. They were divided into 2 groups (the study group, which included 22 patients with periodontitis of various degrees of severity; the control group, which included 12 healthy individuals). The dental examination was carried out in a dental office. As a result of the research, it was established that the rs689466 was associated with periodontitis risk under the allelic model and dominant model. Rs1799946 and rs2274756 did not show any association with the disease in the Ukrainian population.

Key words: periodontitis, oral health, genotyping, human beta-defensin-1, matrix metalloproteinase-9, cyclooxygenase-2.

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ПОЛІМОРФІЗМ ГЕНІВ DEFB1, MMP9 ТА COX2 ПРИ ПАРОДОНТИТІ

Дослідження присвячено пошуку асоціації однонуклеотидних поліморфізмів rs1799946–52G>A гена DEFB1, rs2274756–8202 A>G гена MMP9 та rs689466–1195 A>G гена COX2 з пародонтитом в українській популяції. В дослідженнях приймали участь 34 пацієнта. Вони були розподілені на 2 групи (досліджувана група до якої було залучено 22 пацієнти з пародонтитом різного ступеня важкості; контрольна група до якої було залучено 12 здорових індивідуумів). Стоматологічний огляд було проведено в умовах стоматологічного кабінету. В результаті проведених досліджень було встановлено, що поліморфізм rs689466 асоціювався з ризиком пародонтиту в алельній моделі і домінантній моделі. Дослідна та контрольна групи не відрізнялися достовірно щодо розподілу частот генотипів та алелей поліморфізмів rs1799946 та rs2274756.

Ключові слова: пародонтит, здоров'я порожнини рота, генотипування, бета-дефензин-1, матриксна металопротеїназа-9, циклооксигеназа-2.

The work is a fragment of the research project "Correction of pathogenetic mechanisms of disorders of carbohydrate and lipid metabolism 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.

Periodontal diseases affect approximately 20–50 % of the world's population, according to various estimates, and the global burden of this public health problem is expected to increase in the future due to the general aging of the population [9, 12]. Periodontitis is a common chronic destructive inflammatory disease that, if inadequately treated, can lead to gradual destruction of the structural components of the tooth-supporting apparatus (cementum, periodontal ligament, alveolar bone, gum tissue) and tooth loss. The main etiological factor of periodontal damage is inflammatory and immune reactions to microbial plaque in the oral cavity and enzymes secreted by microorganisms, such as lipases, proteases, and nucleases. Important individual causal factors that can contribute to the development and exacerbation of periodontitis include lifestyle factors, including a diet low in vitamin C and D and other nutrients [2], smoking [11], various systemic diseases such as cardiovascular, respiratory, osteoporosis, atherosclerosis, or diabetes, as well as genetically determined peculiarities of immune response to microorganisms in dental plaque. According to twin studies, the genetic component of periodontitis in women and men accounts for 39 % and 33 %, respectively, of the total contribution of various factors to the pathogenesis of the disease. Genetic predisposition more often determines the rapidly progressing form of the disease, as well as early cases in adolescents and young adults [10].

Single nucleotide polymorphisms are substitutions/deletions/insertions of single nucleotides in the DNA chain, which, although not always leading to a substitution of amino acids in the synthesized protein, can affect the function and expression of genes. According to some estimates, several dozen single nucleotide polymorphisms may contribute to multifactorial diseases, including periodontitis [5]. Recent studies have revealed the role of gene polymorphisms involved in the inflammatory response and immune response, as well as genes encoding a range of other factors, including metalloproteinases, in the progression of periodontitis and the clinical outcome of the disease [4, 6].

The purpose of the study was to investigate the association of single nucleotide polymorphisms rs1799946–52G>A of the DEFB1 gene, rs2274756–8202A>G of the MMP9 gene, and rs689466–1195A>G of the COX2 gene with periodontitis in the Ukrainian population.

Materials and methods. The study involved 34 patients aged 25–55 years. The study group consisted of 22 patients with periodontitis of varying severity; 12 healthy individuals were involved in the control group. Dental examination was conducted in the dental office at the Department of Epidemiology and Prevention of Major Dental Diseases, Pediatric Dentistry and Orthodontics of the SE "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (SE "ISMFS NAMS").

DNA isolation from buccal epithelial cells was performed according to a modified method using Chelex [14]. 200 μl of 5 % solution of Chelex 100 in distilled sterile water (Chelex in sodium form, 100–200 mesh, Bio-Rad) was added to a tube (Eppendorf) containing an applicator with a scraping of epithelial cells. Before adding the resin was mixed in a homogeneous state with a wide-bore pipette and an aliquot was taken directly during mixing. Incubated at 56° for 30 minutes with constant stirring on a thermoshaker. Then incubated at 96°C for 8 minutes, shaking occasionally. After incubation, centrifuge for 3 minutes at 12000 g (Eppendorf Centrifuge 5424). DNA concentration and purity were determined spectrophotometrically (Nanophotometr, Implen, Germany) by taking a 5 μl aliquot directly from the tube with the DNA solution. For polymerase chain reaction (PCR), 5 μl of the supernatant was taken. Allelic variants of polymorphisms rs1799946 DEFB1–52G>A, rs2274756 MMP9–8202 A>G та rs689466–1195 COX2 A>G were assessed by allele-specific PCR. The incubation mixture was prepared under sterile conditions in a PCR box using a PCR buffer from Fermentas (Lithuania). Amplification of the studied regions of the genes was carried out in parallel in two test tubes (Eppendorf) for the normal and mutant allele of each gene in 20 μl of a buffer solution with the addition of 100 nM of each pair of allele-specific oligonucleotide primers (Metabion, Germany), on the "Analytik Jena" thermal cycler (Flex Cycler, Germany). Fractionation of amplification products was carried out by electrophoresis in a horizontal 2 % agarose gel prepared on a disposable Tris-acetate buffer (1xTAE) at a voltage of 100V for 45 minutes. pUC19: Msp1 DNA was used as a molecular weight marker. The agarose gel was stained with ethidium bromide and visualized in ultraviolet light.

Statistical processing of the obtained results, including the test for deviation from the Hardy-Weinberg equilibrium (HWE) and the assessment of the association of genotypes and alleles with the risk of periodontitis by the Pearson $\chi 2$ method, was carried out using the DeFinetti genetic statistics program on the website of the Institute of Genetics (Munich, Germany). Associations were characterized by odds ratio (OR) with 95 % confidence interval and Pearson's $\chi 2$ test. The difference was considered to be statistically significant at $p<0.05$.

Results of the study and their discussion. We conducted genotyping of the rs1799946 DEFB1–52G>A, rs2274756 MMP9–8202A>G, and rs689466–1195 COX2 A>G polymorphisms in a group of patients with periodontitis of varying severity and in a control group. In the investigated groups, we analyzed the distribution of genotype frequencies, the correspondence of their distribution to Hardy-Weinberg equilibrium (HWE), as well as differences between groups in the distribution of genotype and allele frequencies. In the patient group, a significant deviation of the genotype frequency distribution of the rs2274756 MMP9–8202A>G polymorphism from HWE was observed, p=0.003 (Table 1). For the other polymorphisms, genotype distribution frequencies corresponded to the theoretically calculated HWE in both groups (p>0.05, Table 1).

The groups under investigation differed in the distribution of genotypes and alleles of the single nucleotide polymorphism of the COX2 gene rs689466–1195 A>G. The frequency of the mutant G allele of this polymorphism was higher in the patient group compared to the control: 0.273 vs 0.042. This allele was associated with an increased risk of developing periodontitis in the allele model G vs A: p=0.024, odds ratio (OR)=8.625 (95 % CI 1.047–71.083). The presence of the A allele in the hetero- (AG) or homozygous (GG) state significantly increased the risk of periodontitis, corresponding to the dominant inheritance model AG+GG vs AA: p=0.027, OR=9.167 (95 % CI 1.003–83.766).

The patient group and control group did not differ significantly in the distribution of genotypes and alleles of the rs2274756 MMP9–8202 A>G polymorphism. The frequency of the G allele was 0.455 in cases of disease and 0.250 in controls, respectively, p=0.097, and none of the association models with the risk of periodontitis were statistically significant (Table 2).

No differences were found between the studied groups in the distribution of alleles and genotypes of the rs1799946 DEFB1–52G>A polymorphism: the frequency of the A allele was 0.159 in the patient group and 0.083 in the control group, p=0.475.

Table 1

Distribution and comparison of frequencies of genotypes and alleles of rs689466 COX2–1195 A>G та rs2274756 MMP9–8202 A>G polymorphisms in patient groups

Polymorphism	rs689466 COX2–1195 A>G					
Genotype, allele	AA	AG	GG	Alele A	Alele G	HWE p-value
Case, frequency	0.545	0.364	0.091	0.727	0.273	0.696
Control, frequency	0.917	0.083	0.000	0.958	0.042	0.835
Comparison of frequencies	G<>A	AG<>AA	AG+GG<>AA *DM*	GG<>AA+AG *RM*	–	–
OR (95 % CI)	**8.625 (1.047–71.083)**	7.333 (0.785–68.476)	**9.167 (1.003–83.766)**	4.600 (0.199–106.299)	–	–
$\chi 2$ p-value	**0.024**	0.054	**0.027**	0.191	–	–
Polymorphism	rs2274756 MMP9–8202 A>G					
Genotype, allele	AA	AG	GG	Alele A	Alele G	HWE p-value
Case, frequency	0.455	0.182	0.363	0.545	0.455	**0.003**
Control, frequency	0.666	0.167	0.167	0.750	0.250	0.090
Comparison of frequencies	G<> A	AG<>AA	AG+GG<>AA *DM*	GG<>AA+AG *RM*	–	–
OR (95 % CI)	2.500 (0.834–7.496)	1.600 (0.231–11.082)	2.400 (0.555–10.381)	3.200 (0.525–19.495)	–	–
$\chi 2$ p-value	0.097	0.633	0.236	0.196	–	–

Note. CI – confidence interval; DM – dominant model; RM – recessive model; HWE – Hardy-Weinberg equilibrium. Significant values of the odds ratio (95 % CI) and values of p<0.05 are highlighted in bold.

Table 2

Distribution and comparison of frequencies of genotypes and alleles of rs1799946 DEFB1–52G>A polymorphism in patient groups

Polymorphism	rs1799946 DEFB1–52G>A					
Genotype, allele	GG	GA	AA	Alele G	Alele A	HWE p-value
Case,frequency	0.682	0.318	0.000	0.841	0.159	0.375
Control, frequency	0.833	0.167	0.000	0.917	0.083	0.753
Comparison of frequencies	A<>G	GA<>GG	GA+AA <> GG *DM*	AA<>GG+GA *RM*	–	–
OR (95 % CI)	2.081 (0.397–10.920)	2.333 (0.400–13.609)	2.333 (0.400–13.609)	0.677 (0.012–36.891)	–	–
$\chi 2$ p-value	0.475	0.338	0.338	1.000	–	–

Note. CI – confidence interval; DM – dominant model; RM – recessive model; HWE – Hardy-Weinberg equilibrium. Significant values of the odds ratio (95 % CI) and values of p<0.05 are highlighted in bold.

The COX2 gene, also known as PTGS2 (prostaglandin-endoperoxide synthase 2), is located on the short arm of chromosome 1 at 1q25.2–q25.3 and encodes cyclooxygenase-2 (COX2), which is involved in the synthesis of prostaglandins and is activated during inflammation. COX2 is expressed in macrophages, synoviocytes, fibroblasts, smooth muscle cells, chondrocytes, and endothelial cells after specific cytokine induction. Increased expression of COX2 has been observed in gum tissue during periodontitis. However, gingipain proteases produced by *Porphyromonas gingivalis*, one of the key microorganisms in the pathogenesis of periodontitis, can also induce COX2 expression, contributing to the development of inflammatory reactions in periodontal tissues [8]. Previously, it has been shown that single nucleotide polymorphisms in the promoter region of the COX2 gene are associated with the risk of developing periodontitis in Asian patients, with the A allele of the rs689466–1195 COX2 A>G polymorphism being associated with the risk of transitioning periodontitis into a severe chronic form. In a later study of a population of patients of Northern European origin (Germany and the Netherlands), this association was not confirmed. However, another promoter polymorphism of the COX2 gene, rs6681231, showed a link with the risk of aggressive but not chronic periodontitis. In our study, a significant association was found between the rs689466–1195 COX2 A>G polymorphism and the risk of developing periodontitis in the Ukrainian population. MMP-9, which is encoded by the MMP9 gene located on the 20q13.12 region of chromosome 20, is a member of the matrix metalloproteinase family – extracellular zinc-dependent endopeptidases that play an important role in remodeling and degrading

all types of extracellular matrix proteins. Degradation of extracellular matrix proteins by proteases is a key feature of periodontal diseases and can occur as a result of the activity of microorganisms in dental plaque as well as host immune responses. Elevated levels of MMP-9 have been reported in the gingival crevicular fluid in periodontitis [7]. Rs2274756 is associated with changes in serum MMP-9 levels in cardiovascular diseases. In our study, we did not find any association between the rs2274756 MMP9–8202 A>G polymorphism and periodontitis in the Ukrainian population, which may be due to the need for analysis of a larger number of patients. Defensins are proteins secreted by the immune system that have a wide range of antimicrobial activity against bacteria, fungi, and some viruses. The level of beta-defensin-1 protein and DEFB1 gene mRNA concentration was found to be increased in chronic periodontitis [3, 13], however, DEFB1 expression was reduced in cases of aggressive periodontitis and gingivitis [3]. The rs1799946 polymorphism, which is analyzed in our study, has been associated with chronic apical periodontitis in the Brazilian population. However, according to a meta-analysis [1], only the DEFB1 rs1047031 polymorphism, but not rs1799946 DEFB1–52G>A, was associated with the risk of oral diseases, including periodontitis, which is consistent with the results obtained in our study regarding the rs1799946 polymorphism.

Conclusions

1. The rs689466–1195 A>G polymorphism in the promoter region of the COX2 gene, which encodes COX2, was associated with an increased risk of developing periodontitis in both the allelic (G vs A allele) and dominant (AG+GG vs AA genotype) inheritance models. This polymorphism may be one of the markers of genetic susceptibility to periodontitis in the Ukrainian population.

2. The association of polymorphisms of the matrix metalloproteinase-9 gene MMP9 rs2274756–8202 A>G and the human beta-defensin-1 gene DEFB1 rs1799946–52G>A with the risk of periodontitis could not be established.

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