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ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISMS OF GENES IL17A AND TLR2 IN PATIENTS WITH PERIODONTITIS

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The study is devoted to finding the association of single-nucleotide polymorphisms of the IL17A gene and the TLR2 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 rs2275913 polymorphism was associated with the risk of periodontitis in the allelic model and the dominant model. The experimental and control groups did not differ significantly in the frequency distribution of genotypes and alleles of the rs5743708 TLR2 G>A (Arg753Gln) polymorphism.

Key words: oral health, genotyping, interleukin-17A, mediator of immune response.

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АНАЛІЗ ОДНОНУКЛЕОТИДНИХ ПОЛІМОРФІЗМІВ ГЕНІВ IL17A ТА TLR2 У ХВОРИХ НА ПАРОДОНТИТ

Дослідження присвячено пошуку асоціації однонуклеотидних поліморфізмів гена IL17A та гена TLR2 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 rs2275913 асоціювався з ризиком пародонтиту в алельній моделі і домінантній моделі. Дослідна та контрольна групи не відрізнялися достовірно щодо розподілу частот генотипів та алелей поліморфізму rs5743708 TLR2 G>A (Arg753Gln).

Ключові слова: здоров'я порожнини рота, генотипування, інтерлейкін-17А, медіатор імунної реакції.

The study 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.

Periodontitis is a group of inflammatory diseases caused primarily by the interaction of the host organism with the microbiome of the oral cavity under the influence of environmental factors. Smoking, socioeconomic factors, systemic inflammatory diseases (diabetes and others), stress, obesity, and genetic factors are among the risk factors for developing periodontitis [1, 11]. The speed of development of the disease can vary in different individuals. Genetic susceptibility to the disease is more characteristic of an aggressive and rapidly progressing form of the disease [7]. In a number of studies, attempts have been made to establish the relationship between the polymorphism of specific genes, gene expression and various forms of periodontitis. In particular, the study of the association with periodontitis of polymorphisms of genes involved in the inflammatory reaction and immune response, including genes of histocompatibility antigens, IgG class antibodies and their receptors, as well as genes of CD14 molecules, toll-like receptors, vitamin D receptors and other cell receptors. Similar studies also included analysis of the role of polymorphisms in genes encoding metalloproteinases and other enzymes [3]. Much attention was paid to the polymorphism of genes encoding pro- and anti-inflammatory cytokines. The association of chronic and/or aggressive periodontitis with a number of polymorphisms in the IL–1α and IL–1β, IL–2, 4, 6 and 10 interleukin genes was revealed. It was noted that the distribution of polymorphism frequencies can vary significantly in different populations, which does not always allow directly extrapolate data obtained in one population to another population [5].

The purpose of the study was to search for the association of single-nucleotide polymorphisms rs2275913–197G>A of the IL17A gene and rs5743708 G>A (Arg753Gln) of the TLR2 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 rs2275913 IL17A-197G>A and rs5743708 TLR2 G>A (Arg753Gln) 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 statistically significant at $p<0.05$.

Results of the study and their discussion. We performed genotyping of rs2275913 IL17A–197G>A and rs5743708 TLR2 G>A (Arg753Gln) polymorphisms in a group of patients with periodontitis of varying degrees of severity and in a control group. In the studied groups, the distribution of genotype frequencies, compliance of their distribution with the Hardy-Weinberg equilibrium, as well as differences between groups regarding the distribution of genotype and allele frequencies were analyzed. For both polymorphisms, the genotype distribution frequencies corresponded to the theoretically calculated HWE in both groups ($p>0.05$; Table 1).

Table 1

Distribution and comparison of frequencies of genotypes and alleles of rs2275913 IL17A–197G>A polymorphism in patient groups

Polymorphism	rs2275913 *IL17A*–97G>A					
Genotype, allele	GG	GA	AA	Alele G	Alele A	HWE p-value
Case, frequency	0.363	0.455	0.182	0.591	0.409	0.779
Control, frequency	0.750	0.250	0.000	0.875	0.125	0.512
Comparison of frequencies	A<>G	GA<>GG	GA+AA<>GG *DM*	AA<>GG+GA *RM*	–	–
OR (95 % CI)	**4.846 (1.255–18.708)**	3.750 (0.754–18.641)	**5.250 (1.093–25.211)**	10.059 (0.469–215.558)	–	–
$\chi 2$, p-value	**0.015**	0.098	**0.031**	0.054	–	–

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 studied groups differed in the distribution of genotypes and alleles of the single nucleotide polymorphism of the IL17A gene rs2275913–197G>A. The frequency of the mutant allele A of this polymorphism was higher in the group of patients compared to controls: 0.409 and 0.125, respectively. This allele was associated with an increased risk of developing periodontitis: OR=4.846 (95 % CI 1.255–18.708), the reliability of the $\chi 2$ value $p=0.015$. The presence of the A allele in the hetero- (GA) or

homozygous (AA) state determined the increased risk of periodontitis, which corresponds to the dominant model of inheritance GA+AA<>GG: OR=5.250 (95 % CI 1.093–25.211), the reliability of the $\chi 2$ value p=0.015. Rs2275913–197G>A is the most studied promoter polymorphism of the IL17A gene. The group of patients and the control group did not differ significantly in terms of the frequency distribution of genotypes and alleles of the rs5743708 TLR2 G>A (Arg753Gln) polymorphism (p>0.05; Table 2).

Table 2

Distribution and comparison of frequencies of genotypes and alleles of rs5743708 TLR2 G>A (Arg753Gln) polymorphism in patient groups

Polymorphism	rs5743708 TLR2 G>A (Arg753Gln)					
Genotype, allele	GG	GA	AA	Alele G	Alele A	HWE p-value
Case, frequency	0.864	0.136	0.000	0.932	0.068	0.639
Control, frequency	0.917	0.083	0.000	0.958	0.042	0.835
Comparison of frequencies	A<>G	GA<>GG	GA+AA<>GG *DM*	AA<>GG+GA *RM*	–	–
OR (95 % CI)	1.683 (0.165–17.126)	1.737 (0.160–18.802)	1.737 (0.160–18.802)	0.590 (0.011–31.786)	–	–
$\chi 2$ p-value	1.000	1.000	0.646	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 frequency of the mutant A-allele was low both in disease cases and in controls: 0.068 and 0.042, respectively, p=1.000, OR=1.683 (95 % CI 0.165–17.126), while no AA homozygotes were detected in the studied groups.

Increased expression of IL17A has been noted in various inflammatory and autoimmune human diseases [2]. The A-allele of the rs2275913 IL17A–197G>A polymorphism is associated with the risk of stomach cancer, ulcerative colitis, cardiovascular and other diseases [4, 8]. A meta-analysis of the role of gene polymorphisms of various cytokines in the pathogenesis of periodontitis [15] revealed a weak association of the rs2275913 IL17A–197G>A polymorphism with the inflammatory reaction and the risk of periodontitis development. Contradictory data were obtained in the Brazilian population: in the study [10] the A-allele was associated with the risk of chronic periodontitis, while in other studies the association of the A+ genotype with the absence of the disease was found [9], or this polymorphism was not associated with the pathogenesis of periodontitis [13]. Our study revealed a reliable association of the A-allele and A+ genotype with the risk of periodontitis in the Ukrainian population. TLR2 encodes toll-like receptor 2, which is a mediator of the immune response as a receptor for peptidoglycans and lipoteichoic acid of the cell wall of gram-negative bacteria, as well as an unidentified fragment of the cell wall of Porphyromonas gingivalis, one of the key bacteria in the pathogenesis of periodontitis [12]. Currently, 10 toll-like receptors encoded by different TLR genes have been identified. A recent study found an association with periodontitis of two TLR2 polymorphisms: rs1898830 in allelic, recessive, and codominant inheritance patterns and rs5743708 in allelic, dominant, and codominant inheritance patterns in patients of Asian descent, the latter of which was also the subject of our study [6]. At the same time, rs5743708 was not associated separately with chronic or aggressive form of the disease. In addition, the association with chronic periodontitis of the rs7873784 polymorphism of the TLR4 gene in the Asian population was revealed. However, in our study, we failed to detect an association of the rs5743708 TLR2 G>A (Arg753Gln) polymorphism with periodontitis, which is probably due to the low frequency of the A-allele in the Ukrainian population and the need to analyze a larger number of patients.

Conclusions

1. The rs2275913–197G>A polymorphism in the promoter region of the IL17A gene, which encodes the pro-inflammatory cytokine interleukin-17, was associated with the risk of developing periodontitis in the Ukrainian population in allelic (A vs G) and dominant (GA+AA vs GG) inheritance models.

2. It was not possible to detect a connection with the risk of periodontitis of the toll-like receptor 2 gene polymorphism rs5743708 TLR2 G>A (Arg753Gln), which may be due to the low frequency of the A-allele in the Ukrainian population.

References

1. Brodzikowska A, Górski B. Polymorphisms in Genes Involved in Inflammation and Periodontitis: A Narrative Review. Biomolecules. 2022;12(4):552. DOI: 10.3390/biom12040552
2. Chen J, Yang S, Li W, Yu W, Fan Z, Wang M, et al. IL-17A Secreted by Th17 Cells Is Essential for the Host against Streptococcus agalactiae Infections. J Microbiol Biotechnol. 2021;31(5):667–675. DOI: 10.4014/jmb.2103.03053
3. da Silva FRP, Pessoa LDS, Shin JI, Alves EHP, Koga RS, Smith CV, et al. Polymorphisms in the interleukin genes and chronic periodontitis: A field synopsis and revaluation by Bayesian approaches. Cytokine. 2021; 138:155361. DOI: 10.1016/j.cyto.2020.155361
4. Geng GY, Liu HL, Zhao YJ, Wu L, Mao L, Ba N. Correlation between polymorphisms in the IL-17A and IL-17F genes and development of coronary artery disease. Genet Mol Res. 2015;14(3):11488–94. DOI: 10.4238/2015.September.25.15
5. Jia XW, Yuan YD, Yao ZX, Wu CJ, Chen X, Chen XH, et al. Association between IL-4 and IL-4R Polymorphisms and Periodontitis: A Meta-Analysis. Dis Markers. 2017; 2017:8021279. DOI: 10.1155/2017/8021279
6. Jin SH, Guan XY, Liang WH, Bai GH, Liu JG. TLR4 polymorphism and periodontitis susceptibility: A meta-analysis. Medicine (Baltimore). 2016;95(36): e4845. DOI: 10.1097/MD.0000000000004845
7. Li W, Zhu Y, Singh P, Ajmera DH, Song J, Ji P. Association of Common Variants in MMPs with Periodontitis Risk. Dis Markers. 2016; 2016:1545974. DOI: 10.1155/2016/1545974
8. Liu X, Li H. A Systematic Review and Meta-Analysis on Multiple Cytokine Gene Polymorphisms in the Pathogenesis of Periodontitis. Front Immunol. 2022; 12:713198. DOI: 10.3389/fimmu.2021.713198
9. Rimachi Hidalgo MA, Cirelli T, da Silva BR, Nicchio IG, Nepomuceno R, Orrico SRP, et al. Polymorphisms and haplotypes in the Interleukin 17 Alfa gene: potential effect of smoking habits in the association with periodontitis and type 2 diabetes mellitus. Mol Biol Rep. 2021 Feb;48(2):1103–1114. DOI: 10.1007/s11033-021-06172-9
10. Saraiva AM, Alves e Silva MR, Correia Silva Jde F, da Costa JE, Gollob KJ, Dutra WO, et al. Evaluation of IL17A expression and of IL17A, IL17F and IL23R gene polymorphisms in Brazilian individuals with periodontitis. Hum Immunol. 2013 Feb;74(2):207–14. DOI: 10.1016/j.humimm.2012.10.026
11. Savchuk OV, Hasiuk NV, Klytynska OV, Yeroshenko GA, Zaliznyak MS. Clinical and morphological aspects of complex treatment of generalized periodontitis. World of Medicine and Biology. 2020;2(72):115–119. DOI: 10.26724/2079-8334-2020-2-72-115-119
12. Shan C, Aisaiti A, Wu ZP, Wang TT, Zhao J. Association of TLR-2 Gene Polymorphisms with the Risk of Periodontitis: A Meta-Analysis. Dis Markers. 2020; 2020:9353958. DOI: 10.1155/2020/9353958
13. Song B, Zhang YL, Chen LJ, Zhou T, Huang WK, Zhou X, et al. The role of Toll-like receptors in periodontitis. Oral Dis. 2017;23(2):168–180. DOI: 10.1111/odi.12468
14. Walsh PS, Metzger DA, Higushi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques. 2013 Mar;54(3):134–9. DOI: 10.2144/000114018
15. Zacarias JM, Sippert EÂ, Tsuneto PY, Visentainer JE, de Oliveira e Silva C, Sell AM. The Influence of Interleukin 17A and IL17F Polymorphisms on Chronic Periodontitis Disease in Brazilian Patients. Mediators Inflamm. 2015;2015:147056. DOI: 10.1155/2015/147056

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