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STUDY OF THE ASSOCIATION OF POLYMORPHISMS OF CALCIUM METABOLISM AND BONE HOMEOSTASIS GENES WITH THE RISK OF ATHEROSCLEROSIS IN PATIENTS WITH GENERALIZED PERIODONTITIS

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Genotyping of single nucleotide polymorphisms of several genes of calcium metabolism and bone homeostasis in patients with generalized periodontitis on the background of atherosclerosis atherosclerosis and in the control group was carried out. The association of the A allele of the rs6252 polymorphism of the PTH (parathyroid hormone) gene with the risk of atherosclerosis was revealed: OR=3.857 (95 % CI 1.243-11.968), the reliability of the $\chi 2$ value p=0.017. The homozygous AA genotype of this polymorphism was also associated with an increased risk of atherosclerosis in a recessive inheritance model (p=0.035). The experimental and control groups did not differ significantly in the distribution of frequencies of genotypes and alleles of polymorphisms rs1544410 of the VDR gene (vitamin D receptor), rs1801725 of the CASR gene (calcium-sensitive receptor), rs1126616 of the SPP1 gene (osteopontin), rs1801197 1377 C>T of the CTR gene (calcitonin receptor) and rs1801133 of the MTHFR gene (methylenetetrahydrofolate reductase).

Key words: atherosclerosis, periodontitis, calcium exchange, bone homeostasis, polymorphism, genotyping.

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ДОСЛІДЖЕННЯ АСОЦІАЦІЇ ПОЛІМОРФІЗМІВ ГЕНІВ КАЛЬЦІЄВОГО ОБМІНУ ТА КІСТКОВОГО ГОМЕОСТАЗУ З РИЗИКОМ АТЕРОСКЛЕРОЗУ У ПАЦІЄНТІВ З ГЕНЕРАЛІЗОВАНИМ ПАРОДОНТИТОМ

Проведено генотипування однонуклеотидних поліморфізмів декількох генів кальцієвого обміну і кісткового гомеостазу у пацієнтів з генералізованим пародонтитом на тлі атеросклерозу і в контрольній групі. Виявлена асоціація алеля А поліморфізму rs6252 гена ПТГ (паратиреоїдного гормону) з ризиком атеросклерозу: ВШ=3,857 (95 % ДІ 1,243-11,968), значення достовірності $\chi 2$ p=0,017. Гомозиготний генотип АА даного поліморфізму також асоціювався з підвищеним ризиком атеросклерозу в рецесивній моделі успадкування (p=0,035). Дослідна і контрольна групи не відрізнялися достовірністю по розподілу частоти генотипів і алелей поліморфізмів rs1544410 гена VDR (рецептор вітаміну D), rs1801725 гена CASR (кальцій-чутливий рецептор), rs1126616 гена SPP1 (остеопонтин), rs1801197 1377 C>T гена CTR (рецептор кальцитоніну) і rs1801133 гена MTHFR (метилентетрагідрофолатредуктаза).

Ключові слова: атеросклероз, пародонтит, кальцієвий обмін, кістковий гомеостаз, поліморфізм, генотипування.

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Atherosclerosis is the most serious and clinically significant form of arteriosclerosis – a broad term encompassing a group of disorders that cause thickening and loss of elasticity of arterial walls. Increased permeability of the endothelial layer to macromolecules permits low-density lipoproteins and triglyceride-rich remnant lipoproteins to accumulate, aggregate, and undergo chemical modifications within the arterial intima, ultimately triggering chronic vascular inflammation [2]. This pathology drives the development of cardiovascular diseases, which rank among the leading causes of mortality worldwide [9].

Risk factors for atherosclerosis include several metabolic disturbances and diseases – hyperlipidaemia and hypercholesterolaemia, hypertension, hyperhomocysteinaemia, hyperuricaemia, diabetes mellitus, and chronic kidney disease – as well as unhealthy diet and obesity, physical inactivity, smoking, excessive psychosocial stress, environmental exposures, and genetic predisposition [7].

Atherosclerosis is also strongly age-dependent: after 50 years in men and 60 years in women, the risk rises sharply, reflecting natural age-related alterations in the circulatory system and lifelong accumulation of risk factors [8].

The genetic component of atherosclerosis largely reflects the additive effects of numerous genes engaged in diverse metabolic pathways that either promote the disease directly or modulate susceptibility to other risk factors. Genome-wide association studies have identified approximately 200 genes associated, to varying degrees, with atherosclerotic risk: 17 genes regulate lipid metabolism, 16 govern mitosis and cellular proliferation, 24 mediate vascular remodelling, 9 participate in angiogenesis and transcription, 14

relate to insulin resistance and diabetes, and 15 modulate inflammatory responses; the functions of a further 76 genes remain undefined [3].

In recent years, research exploring links between periodontal diseases and cardiovascular pathology has expanded, stimulated by the "unified theory of atherogenesis". A 2024 review of 1,572 articles corroborated the association between periodontitis and atherosclerosis and highlighted key mechanisms – chronic inflammation, bacterial dissemination (particularly Porphyromonas gingivalis), and dysregulated immune responses [4].

Identifying novel genetic markers of atherosclerosis and developing multigene diagnostic models based on them could enable early detection of individuals at risk of persistently elevated low-density lipoprotein levels and ensuing vascular pathology [6].

The purpose of the study was to identify, in patients with generalized periodontitis, the association between atherosclerosis and a panel of single-nucleotide polymorphisms in genes governing calcium homeostasis and bone metabolism – specifically, VDR rs1544410 c.IVS7+283, CASR rs1801725 G>T (A986S), SPP1 rs1126616 9250 C>T, PTH rs6252 G>A, CTR rs1801197 1377 C>T, and MTHFR rs1801133 677 C>T (Ala222Val).

Materials and methods. A total of 38 persons participated in the study – 22 women (57.9 %) and 16 men (42.1 %) – all diagnosed with generalized periodontitis (GP) and aged 59–69 years (mean age 64.95±0.40 years; σ=2.49; Me=65). Regarding the distribution of periodontal disease among the examined patients, 55.3 % (21 patients) had GP grades I–II, whereas 44.7 % (17 persons) exhibited GP grades II or II–III.

The main group comprised 20 persons with GP and confirmed atherosclerosis (7 men, 35 %, and 13 women, 65 %). The control group consisted of 18 persons (9 women and 9 men, 50 % each).

The presence of atherosclerosis in the examined persons was verified by official certificates issued by their family physicians, internists, or cardiologists.

The research was conducted in the molecular genetic laboratory of the State Establishment "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (headed by T.H. Verbytska, PhD).

Genomic DNA was extracted from buccal epithelial cells using a modified Chelex-based protocol [11]. An Eppendorf tube containing the applicator with the scraped epithelial cells was filled with 200 μL of a 5 % Chelex 100 suspension prepared in sterile distilled water (Chelex, sodium form, 100–200 mesh; Bio-Rad). Immediately before use the resin was homogenised with a wide-bore pipette, and the aliquot was taken while mixing. Samples were incubated at 56 °C for 30 min with continuous agitation on a thermoshaker, followed by incubation at 96 °C for 8 min with intermittent vortexing. After heat treatment the samples were centrifuged for 3 min at 12 000 g (Eppendorf Centrifuge 5424). DNA concentration and purity were assessed spectrophotometrically (Nanophotometer, Implen) by sampling 5 μL directly from the DNA solution. A 5 μL aliquot of the supernatant was used for polymerase chain reaction (PCR).

Allelic variants of the polymorphisms VDR rs1544410 c.IVS7+283 G>A, CASR rs1801725 G>T (A986S), SPP1 rs1126616 9250 C>T, PTH rs6252 G>A, CTR rs1801197 1377 C>T, and MTHFR rs1801133 677 C>T (Ala222Val) were identified by allele-specific triple-primer PCR employing two forward primers targeting the 5′ region (one specific for the mutant allele, the other for the wild-type allele) and a common reverse primer situated in the 3′ region. Amplification was performed on a Flex Cycler thermal cycler (Analytik Jena, Germany) in parallel Eppendorf tubes – one for the wild-type and one for the mutant allele of each gene – in a 20 μL reaction mixture containing PCR buffer (Fermentas, Lithuania), 100 nM of each oligonucleotide primer (Metabion, Germany), and 100–150 ng of genomic DNA.

Amplification and restriction products were fractionated by horizontal electrophoresis in a 2 % agarose gel prepared in single-use Tris-acetate buffer (1× TAE) at 100 V for 45 min. DNA pUC19 digested with MspI served as the molecular-weight marker. The gel was stained with ethidium bromide and visualised under ultraviolet illumination.

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 between groups 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). The degree of association of genotype and alleles of patients with leukoplakia, neoplasia and controls was calculated by the value of the odds ratio (OR) with 95 % confidence interval and Pearson's $\chi 2$ test. The difference was considered to be statistically significant at $p<0.05$ [1].

Results of the study and their discussion. Genotyping was carried out for the polymorphisms VDR rs1544410 c.IVS7+283, CASR rs1801725 G>T (A986S), SPP1 rs1126616 9250 C>T, PTH rs6252 G>A, CTR rs1801197 1377 C>T, and MTHFR rs1801133 677 C>T (Ala222Val) in patients with generalized periodontitis (GP) who either had concomitant atherosclerosis or were free of cardiovascular pathology. Genotype-frequency distributions in both cohorts were examined for conformity to Hardy-Weinberg equilibrium (HWE), and inter-group differences in genotype and allele frequencies were assessed. Only the CC genotype of the SPP1 rs1126616 9250 C>T variant was observed in both groups; accordingly, this polymorphism was excluded from further analysis.

Distribution and comparative analysis of genotype and allele frequencies for the polymorphisms VDR rs1544410 c.IVS7+283 and CASR rs1801725 G>T in individuals with generalized periodontitis stratified by the presence of atherosclerosis presented in Table 1.

Table 1

Distribution and comparative analysis of genotype and allele frequencies for the polymorphisms VDR rs1544410 c.IVS7+283 and CASR rs1801725 G>T in individuals with generalized periodontitis stratified by the presence of atherosclerosis

Genotype, allele	Main group, frequency	Control group, frequency	Comparison of frequencies	OR (95 % CI) oncology-control	χ2 p-value
rs1544410 VDR c.IVS7+283 G>A					
GG	0.400	0.400	A<>G	1.206 (0.516-2.816)	0.665
GA	0.350	0.440	GA<>GG	0.795 (0.211-3.000)	0.735
AA	0.250	0.160	GA+AA<>GG DM	1.000 (0.301-3.321)	1.000
Alele G	0.575	0.620	AA<>GG+GA RM	1.562 (0.312-7.819)	0.590
Alele A	0.425	0.380	–	–	–
HWE p-value	0.204	0.696	–	–	–
rs1801725 CASR G>T (A986S)					
GG	0.600	0.500	T<>G	0.750 (0.210-2.683)	0.743
GT	0.400	0.500	GT<>GG	0.667 (0.145-3.075)	0.602
TT	0.000	0.000	GT+TT<>GG DM	0.667 (0.145-3.075)	0.602
Alele G	0.800	0.750	TT<>GG+GT RM	0.440 (0.008-25.159)	1.000
Alele T	0.200	0.250	–	–	–
HWE p-value	0.264	0.292	–	–	–

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.

No statistically significant inter-group differences were observed in genotype or allele distributions for the VDR rs1544410 c.IVS7+283 and CASR rs1801725 G>T polymorphisms analysed.

Distribution and comparative analysis of genotype and allele frequencies for the polymorphisms PTH rs6252 G>A, CTR rs1801197 1377 C>T, and MTHFR rs1801133 677 C>T (Ala222Val) in individuals with generalized periodontitis stratified by the presence of atherosclerosis presented in Table 2.

For the remaining loci, genotype frequencies agreed with HWE expectations in both cohorts (p>0.05), except for rs1801197 CTR 1377 C>T in the atherosclerosis group, which deviated from equilibrium (p=0.016).

A significant difference was detected between the atherosclerosis group and the control group in the distribution of genotypes and alleles for the PTH rs6252 G>A single-nucleotide polymorphism. The frequency of the mutant A allele was higher among patients with atherosclerosis than in controls (0.675 vs 0.350, respectively). Accordingly, the A allele was significantly associated with an increased risk of atherosclerosis (odds ratio=3.857; 95 % CI 1.243–11.968; χ^2 p=0.017). Homozygosity for the A allele (AA) also conferred an elevated risk under a recessive model (AA vs GG+GA), with p=0.035 and a high, though not statistically significant, odds ratio (OR=11.000; 95 % CI 0.928–130.324).

No statistically significant inter-group differences were observed in genotype or allele distributions for the remaining polymorphisms analysed. Notably, homozygotes for the wild-type C allele of CTR rs1801197 1377 C>T were absent in both groups. In the atherosclerosis cohort, heterozygotes (CT) predominated (frequency=0.700), whereas in the control cohort, TT homozygotes were more frequent (frequency=0.600); however, these differences did not reach statistical significance.

Table 2

Distribution and comparative analysis of genotype and allele frequencies for the polymorphisms PTH rs6252 G>A, CTR rs1801197 1377 C>T, and MTHFR rs1801133 677 C>T (Ala222Val) in individuals with generalized periodontitis stratified by the presence of atherosclerosis

Main group, frequency	Control group, frequency	Comparison of frequencies	OR (95 % CI) oncology-control	χ2 p-value	Genotype, allele
rs6252 PTH G>A					
GG	0.200	0.500	A<>G	3.857 (1.243-11.968)*	0.017
GA	0.250	0.400	GA<>GG	1.000 (0.156-6.420)	1.000
AA	0.550	0.100	GA+AA<>GG DM	2.667 (0.500-14.217)	0.243
Alele G	0.325	0.650	AA<>GG+GA RM	11.000 (0.928-130.324)	0.035*
Alele A	0.675	0.350	–	–	–
HWE p-value	0.054	0.755	–	–	–
rs1801197 CTR 1377 C>T					
CC	0.000	0.000	T<>C	0.464 (0.130-1.660)	0.232
CT	0.700	0.400	CT<>CC	3.222 (0.056-186.825)	1.000
TT	0.300	0.600	CT+TT<>CC DM	1.952 (0.036-105.522)	1.000
Alele C	0.350	0.200	TT<>CC+CT RM	1.000 (0.017-58.434)	1.000
Alele T	0.650	0.800	–	–	–
HWE p-value	**0.016**	0.429	–	–	–
rs1801133 MTHFR 677 C>T (Ala222Val)					
CC	0.600	0.450	T<>C	1.000 (0.375-2.669)	1.000
CT	0.250	0.550	CT<>CC	0.341 (0.087-1.336)	0.112
TT	0.150	0.000	CT+TT<>CC DM	0.545 (0.155-1.914)	0.342
Alele C	0.725	0.725	TT<>CC+CT RM	5.320 (0.244-115.863)	0.152
Alele T	0.275	0.275	–	–	–
HWE p-value	0.095	0.089	–	–	–

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.

Over the past decade, accumulating evidence has confirmed a tight connection between atherosclerosis and periodontitis. A meta-analysis of 15 observational studies encompassing 17 330 participants demonstrated that the presence of periodontitis is associated with a higher likelihood of carotid atherosclerosis (odds ratio ≈ 1.27; 95 % CI 1.14–1.41) [4]. Several pathogenic mechanisms have been proposed: Periodontitis increases circulating concentrations of C-reactive protein (CRP) and pro-inflammatory cytokines (IL-1, IL-6, IL-8, TNF-α), which damage vascular endothelium and promote atherosclerotic plaque formation [5, 14]; Periodontal pathogens – particularly Porphyromonas gingivalis and Treponema denticola – can enter the bloodstream, localise within the vessel wall, and contribute directly to plaque development [4]; Infection-induced inflammation elicits immune responses in the vasculature that accelerate smooth-muscle-cell hyperplasia and intravascular lipid accumulation [4, 5]. Randomised clinical trials have shown that intensive periodontal therapy improves vascular function, lowers CRP, and can even reduce carotid intima-media thickness [4, 12]. Previous studies have linked other PTH gene variants to osteopenia, osteoporosis, and periodontal bone loss. Atherosclerosis and periodontitis are now regarded as chronic inflammatory disorders that share common pathogenic pathways [15]. Among these, genetic factors affecting calcium metabolism and bone remodelling have attracted particular attention. In the present study, we found a statistically significant association between the A allele of the rs6252 PTH polymorphism and an increased risk of atherosclerosis, corroborating current concepts that disturbances in calcium-phosphate homeostasis drive vascular remodelling and calcification [10]. Under systemic inflammatory conditions, parathyroid hormone (PTH) activation enhances bone resorption, stimulates cytokine secretion, and alters endothelial function – mechanisms that may be pivotal in both atherosclerosis and periodontitis. Elevated PTH concentrations have been documented in patients with severe periodontitis, and PTH has been shown to promote osteoclastogenesis and reduce bone density in the alveolar region. Moreover, patients with chronic kidney disease, in whom secondary hyperparathyroidism is common, exhibit a higher prevalence of severe periodontitis, underscoring a pathophysiological interdependence [1]. Molecular data further indicate that PTH induces RANKL expression while lowering OPG levels, thereby stimulating bone resorption in both interdental septa and

calcified atherosclerotic lesions [13]. Consequently, genetic variants that alter PTH gene function or expression may simultaneously influence periodontal bone loss and vascular wall calcification. In our cohort, the remaining polymorphisms (rs1544410 in VDR, rs1801725 in CASR, rs1126616 in SPP1, rs1801197 in CTR, and rs1801133 in MTHFR) did not differ significantly between groups, although literature suggests they can modulate mineral metabolism and inflammatory responses in various populations [10]. Taken together, our findings implicate rs6252 in PTH as a potential shared genetic risk marker for both periodontitis and atherosclerosis, particularly within the context of metabolic-inflammatory disorders.

Conclusion

Thus, only one of the six investigated polymorphisms in genes involved in calcium metabolism and bone homeostasis – PTH rs6252 G>A – was significantly associated with atherosclerosis risk.

The linkage of the rs6252 A allele to atherosclerosis may likewise be pertinent to periodontitis, because parathyroid hormone modulates bone resorption and alveolar bone loss is a cardinal feature of periodontitis. Shared pathophysiological mechanisms – chronic inflammation, calcium dysregulation, and tissue remodelling – support the hypothesis that this SNP could act as a molecular marker conferring increased susceptibility to both disorders.

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