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THE ASSOCIATION OF *APA*I-POLYMORPHISM OF VITAMIN D RECEPTOR GENE *(VDR)* WITH DEVELOPMENT OF GENERALIZED PARODONTITIS IN UKRAINIAN POPULATION

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ABSTRACT

Introduction: At present, it is believed that the genetic component is important in the pathogenesis of periodontitis. One of the candidate genes that are of major importance in the development of the disease is the vitamin D receptor gene (VDR). The association of its genetic polymorphisms, in particular *Apa*I, with periodontitis in different populations of the world is proved.

The aim: To study the association of the *Apa*I-polymorphism *VDR* gene with the development of generalized periodontitis in the Ukrainian population.

Materials and methods: Patient genotypes were determined by polymerase chain reaction with subsequent analysis of restriction fragment length (PCR-RFLP) from buccal epithelium 116 patients with generalized periodontitis (GP) and 67 individuals of control group. Statistical analysis was performed by using SPSS-17,0 program

Results: As a result of the performed studies, it was shown that in the group of patients with GP, the ratio of homozygous for the main allele (a/a), heterozygote (a/A) and homozygote for the minor allele (A/A) was 26 (22,4%), 62 (53,4%), 28 (24,2%), and in control group – 25 (37,3%), 27 (40,3%), 15 (22,4%), respectively. The distribution of genotypes in the comparison groups was not statistically significant (P = 0,084). By the method of binary logistic regression in the framework of the additive inheritance model (a/A vs a/a), a reliable relationship of the genotype with the *Apa*I-polymorphism of the *VDR* gene was established with the development of generalized periodontitis (P=0,029). It was shown that in heterozygotes (a/A) the risk of GP in 2,208 (95% CI = 1,084-4,496) times is higher than in homozygotes of the main allele (a/a). After adjusting for age, sex, smoking habit, BMI, the reliability of these results was maintained (P = 0,030).

Conclusions: The *Apa*I-polymorphism of the *VDR* gene is associated with the development of generalized periodontitis in the Ukrainian population

KEY WORDS: generalized periodontitis, gene polymorphism, *Apa*I, *VDR*

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INTRODUCTION

Periodontal diseases are the main cause of the loss of teeth among adults. It is known that intact periodontium is detected only in 12% of people; others have damaging of various degrees of severity - from initial inflammatory processes to severe destructive changes with the loss of teeth [1]. Today, cases of aggressive current of periodontitis with the progression of resorption of bone tissue of the jawbones at younger ages are becoming more frequent [2]. At present, reliable markers for assessing the predisposition of patients to the onset of periodontitis and its prognosis are not yet found, which makes it difficult to conduct timely preventive measures, early diagnosis of the disease, and the use of individual approaches in treatment. Thus, the most relevant today is the search for methods of diagnosis of periodontal diseases prior to the appearance of its clinical signs. This will avoid the development of complications of the disease, its transition to a more severe form. Moreover, modern molecular genetic methods can become irreplaceable in this matter. An important place among them is the study of the role of single nucleotide polymorphisms of candidate genes in the development of dental diseases.

Since the condition of the dento-facial system is inextricably linked to the general status of bone tissue in the body, recent studies have been conducted on the study of the polymorphism of the genes involved in the regulation of calcium homeostasis. In a number of studies, the relationship between different diseases with a disorder of metabolism of the bone tissue of the body and the pathology of periodontal disease has been demonstrated. Thus, if patients have severe bone mineral density loss, the risk of developing severe forms of generalized periodontitis increases [3], and a decrease in the density of bone tissue in the peripheral skeleton is accompanied by the loss of mineral bone density in the jaws [4].

genes which polymorphis s are associated with changes in the bone tissue, a promising marker today is the vitamin D receptor gene (VDR), which has an effect on the development of periodontitis through the actiononcalcium metabolism, on the one hand, and the immune system on the other [5].

s established the association of the polymorphisms of the VDR gene, including *Apa*I, with diseases such as osteoporosis, urolithiasis, renal osteodystrophy, various neoplasms, cardiovascular diseases, and peri-

odontal diseases [6-11]. The literature on this issue is quite heterogeneous and contradictory, which may be due to the genetic heterogeneity of the samples, as well as to various comparison parameters.

THE AIM

The purpose of this research was to study the association of *Apa*I-polymorphism of the VDR gene with the development of periodontitis among the Ukrainian population.

MATERIALS AND METHODS

The study used buccal epithelium of 116 patients with generalized periodontitis and 67 people without GP. The examination of each patient began with the collection of a common anamnesis (age, gender, body weight index (BWI), bad habits, physical activity, the main and possible risk factors for destructive periodontal disease). A special anamnesis included the information about the availability or absence of complaints of pain, teeth loosening, bleeding of gums, and bad breath. A periodontological examination of patients included instrumental and hardware methods. The clinical attachment loss (CAL), the recession of the gums, and the depth of the periodontal pocket were determined with the help of the periodontal broach. Based on these data, the patients were divided into two groups, the control group and the main one. The main group was made up of the patients with generalized periodontitis of a chronic course. Depending on the degree of severity, the main group was divided into 3 subgroups according to the clinical attachment loss: mild case - 1-2 mm CAL, average - 3-4 mm CAL, severe - more than 5 mm of CAL (American Academy of Periodontology (recommended by the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions). The panoramic X-ray images were characterized by the complete destruction of the bone tissue, based on which the severity of the disease was confirmed.

rk was performed in accordance with the principles of the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with the participation of a person as a research object" and approved by the Bioethics Commission of the Medical Institute of Sumy State University. Before entering the study, all the participants provided written informed consent for the use of biological material in genetic research.

ge g, the buccal epithelium of the patients was collected with a special broach from the inner surface of the cheeks. The broaches were placed in 1.5 ml Eppendor tubes and stored at -20 ° C. DNA from the buccal epithelium was isolated using the NeoPrep50 DNA Magnet commercial kit ("NEOGEN", Ukraine) according to the producer's protocol.

on that contained the *Apa*I site was amplified using a pair of specific primers: forward 5'-CAGAGCATGGACAGGGAGCAA-3' and reverse 5'-CACTTCGAGCACAAGGGGCGTTAGC-3' (Metabion, Germany). The amplification mixture consisted of 50-100 ng DNA, 5 μl 5x PCR buffer, 1.5mmol magnesium sulfate, 150 μm mixture of four nucleotide triphosphates, 15 pM each of the primers and 0.75 ED Taq-polymerase (Thermo Scientific, USA). The volume of the mixture was adjusted to 25 μl with deionized water. Polymerase chain reaction (PCR) was performed in the GeneAmp PCR System 2700 thermocycler (Applied Biosystems, USA). The amplification consisted of 35 cycles: 1 cycle 94 ° C (4 min), 2 to 34 cycle denaturation - 94 ° C (50 s), primer hybridization - 64.5 ° C (45 s) and elongation - 72 ° C (1 min), 35 cycle - 72 ° C (5 min). Then, 6 μl of the amplification product was incubated at 37 ° C for 20 hours with 5 ED restriction enzymes of *Apa*I (Thermo Scientific, USA) in buffer B of the following composition: 10 mM Tris-HCl (pH 7.5), 10 mM magnesium chloride, and 0.1 mg / ml of albumin. If the VDR gene at position 59979 was guanine, the amplificator, which consisted of 501 base pairs, was cleaved by the restriction enzyme *Apa*I into two fragments – 284 and 217 base pairs. When the guanine was replaced with thiamine, the site of restriction for *Apa*I disappeared, and a fragment of 501 size of a base pair formed (Fig. 1).

ion of ampl icators and restriction products was performed using a 2.5-agar gel (Sigma-Aldrich, USA) horizontal electrophoresis, which contained ethidium bromide (Sigma-Aldrich, USA). Horizontal electrophoresis (0,1A; 140V) is performed for 30 minutes. Visualization of DNA after electrophoresis is carried outusing the automatic video-reading system "Vi-Tran" in a transilluminator ("Biocom", Russia).

l analysis was performed using the SPSS-17 program. The relevance of Harry-Weinberg equilibrium genotype distribution was checked using the Online Encyclopedia for Genetic Epidemiology Studies (http://www.oege.org/software/hwe-mr-calc.shtml). To determine the validity of the difference between the two samples was performed using the student's t-criterion. To compare the distribution of genotypes in the study and control groups, the Pearson χ^2 criterion was used. All tests were bilateral, values $P<0,05$ were considered statistically significant.

RESULTS AND DISCUSSION

As a result of genotyping the patients in the groups of comparisons with the *Apa*I-polymorphism of the VDR gene and the statistical processing of the data obtained, the frequency of the genotypes and alleles they occur among the Ukrainian population was established, and the correspondence between the distribution of the main and minor alleles of the Hardy-Weinberg equilibrium (Table I) was verified. In the group of patients with GP, the ratio of homozygotes to the main allele (a/a), heterozygote (a/A) and homozygote for the minor allele (A/A) was 26 (22,4%), 62 (53,4%) and 28 (24,2%), while in the control it was 25 (37,3%), 27 (40,3%) and 15 (22,4%) respectively, which did not have statistical significance ($P = 0,084$). The frequency of the primary (a) and minor (A) alleles was among the patients 0,491 to 0,509, and among the healthy – 0,575 to 0,425.

stage of the analysis of the results, the patients of the control and the main groups were divided into subgroups,

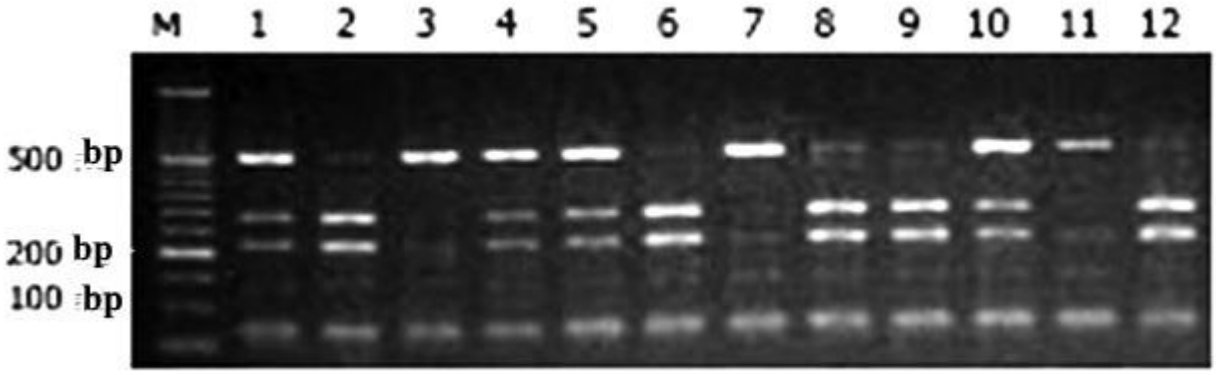

Figure 1. Results of the restriction analysis of *Apa*I-polymorphism of the VDR gene. M - molecular weight marker (in pairs of nucleic bases) of the paths 3,7,11 correspond to the genotype a/a, 1,4,5,10 - genotype a/A, 2,6,8,9,12 genotype A/A.

Table I. Distribution of alleles and genotypes by *Apa*I-polymorphism of the VDR gene in comparison groups

Genotype	Generalized periodontitis (n = 116)		Control (n = 67)		P_{HWE}	P
	n	%	n	%		
Homozygotes a/a	26	22,4	25	37,3		
Heterozygotes a/A	62	53,4	27	40,3		0.084
Homozygotes A/A	28	24,2	15	22,4		
Allele						
a	114	49,1	77	57,5	0.15	0.125
A	118	50,9	57	42,5		

Note. n - the number of patients.

Table II. Distribution of genotypes by *Apa*I-polymorphism of the VDR gene in patients with various risk factors for generalized periodontitis (GP)

Gender				
Genotype	Female		Male	
	GP (−)	GP (+)	GP (−)	GP (+)
a/a, n (%)	13 (34,2)	12 (20,3)	12 (41,4)	14 (24,6)
a/A, n (%)	17 (44,7)	34 (57,6)	10 (34,5)	28 (49,1)
A/A, n (%)	8 (21,1)	13 (22,1)	7 (24,1)	15 (26,3)
	P = 0,291		P = 0,251	

Smoking				
Genotype	Do not smoke		Smoke	
	GP (−)	GP (+)	GP (−)	GP (+)
a/a, n (%)	16 (38,1)	13 (19,7)	9 (36,0)	13 (26,0)
a/A, n (%)	18 (42,9)	38 (57,6)	9 (36,0)	24 (48,0)
A/A, n (%)	8 (19,0)	15 (22,7)	7 (28,0)	13 (26,0)
	P = 0,107		P = 0,566	

Body weight index (BWI)				
Genotype	BWI < 25		BWI ≥ 25	
	GP (−)	GP (+)	GP (−)	GP (+)
a/a, n (%)	16 (34,0)	15 (27,3)	9 (45,0)	11 (18,1)
a/A, n (%)	20 (42,6)	28 (50,9)	7 (35,0)	34 (55,7)
A/A, n (%)	11 (23,4)	12 (21,8)	4 (20,0)	16 (26,2)
	P = 0,675		P = 0,051	

Note. n - the number of patients

Table III. Distribution of genotypes by *Apa*l-polymorphism of the VDR gene in the groups of patients with generalized periodontitis (GP) of various degrees of severity.

Genotype			Degree of severity GP		
			1	2	3
Homozygotes	a/a	n	10	13	3
		%	22.2	22.8	21.4
Heterozygotes	a/A	n	22	34	6
		%	48.9	59.6	42.9
Homozygotes	A/A	n	13	10	5
		%	28.9	17.6	35.7
			P = 0,539		

Note. n - the number of patients

Table IV. Analysis of association of genotypes on *Apa*l-polymorphism of the VDR gene with the risk of development of generalized periodontitis

Model	P_n	OR_n (95% CI)	P_a	OR_a (95% CI)
Dominant	0,032	2,060 (1,065–3,986)	0,036	2,088 (1,048–4,158)
Recessive	0,788	1,103 (0,540–2,254)	0,873	1,062 (0,506–2,230)
Superdominant	0,088	1,701 (0,925–3,129)	0,078	1,771 (0,938–3,347)
Additivea	0,029	2,208 (1,084–4,496)	0,030	2,279 (1,084–4,793)
	0,169	1,795 (0,780–4,131)	0,202	1,758 (0,739–4,181)

Note. 95% CI - 95% confidence interval; P_n - the observed value of P (without correction for covariates); OR_n - observed ratio of odds; P rial hypertension and the habit of smoking;
ORa - odds ratio after correction for covariates; a - the first row in the additive model represents the comparison between the A/a - and a/a-genotypes, the second row is between the A/A- and a/a-genotypes.

according to sex, smoking habit, and body weight index (Table II). There were no differences in the distribution of genotypes in the group with GP and the control among people of different genders; those who smoke and do not smoke have BWI less than 25 kg / m2. However, in the subgroup of the patients with BWI more than 25 kg / m^2, the ratio of the genotypes a/a, a/A and A/A significantly differed from 11 (18,0%), 34 (55,7%) and 16 (26,3%) for the main group and 9 (45,0%), 7 (35,0%) and 4 (20,0%) for control (P = 0,051). Analysis in the subgroups of the patients with generalized periodontitis of various degrees of severity also did not reveal differences in the distribution of genotypes (Table III).

f the regression analysis of the associat on of genotypes on *Apa*I-polymorphism of the VDR gene with the development of GP in the framework of different patterns of inheritance are given in Table. IV. By the method of binary logistic regression, a statistically significant relationship was established within the additive (a/A vs a/a) model of inheritance (P_n = 0,029). The calculation of relative risk within the framework of the presented model showed that the heterozygote (a/A) had a GP risk of 2,208 (95% CI = 1,084-4,496) times higher than homozygotes to the main allele (a/a). After adjusting for age, sex, smoking habit, BWI, the reliability of these results was maintained, the P_a indicator was 0,030 (OR_a = 2,279; 95% CI = 1,084-4,793).

e, which in humans has a length of 63,495 pairs of nucleotides, is represented by one copy and is on the 12th chromosome at position 12q13.11 [12]. The gene consists of 11 exons. Currently, 17,757 of its polymorphisms are known. *Apa*I-polymorphism is localized in the 8th intron near the 3'-UTR region (untranslated region) [13]. The polymorphic variant of *Apa*I is characterized by the replacement of guanine at position 59979 on thymine. Polymorphisms in the introns are not functionally significant, since they do not alter the sequences of nitrogenous bases in the semantic part of the gene. However, being linked to the regulatory regions of the gene, they can act as markers of the functional relationships of other SNPs with the development of pathological processes and diseases. Thus, its association with psoriasis [14], vitiligo [15], asthma [16], osteopenia [17], oncological [18], cardiovascular [10], and other diseases have been proven.

has shown that *Apa*I-polymorphism of the V R gene is associated with the development of generalized periodontitis among the Ukrainian population. Similar results were obtained in the work of other researchers. Inagaki K. et al. studied the distribution of genotypes on *Apal*-polymorphism of the VDR gene in a group of middle-aged men of the United States [19]. The frequency of genotypes according to this polymorphism was in the group under study: a/a – 41 (32,8%), a/A – 58 (46,4%), A/A – 26 (20,8%). These data are not statistically significantly different from the results we obtained (P = 0,51). In addition, the authors concluded that *Apa*I-polymorphism is

associated with alveolar bone loss, clinical attachment loss and the loss of teeth in older men. Naito M. et al. did not find a statistical connection between *Apa*I-polymorphism and the development of periodontitis among the Japanese [20]. Although the authors identified the connection between certain haplotypes and the development of chronic periodontitis. Data on the association of *Apa*I-polymorphism with the development of chronic periodontitis among the Taiwan population have been obtained [21].

CONCLUSIONS

*Apa*I-polymorphism of the VDR gene is associated with the development of generalized periodontitis among the Ukrainian population. In a heterozygote (a/A), the risk of developing the disease is 2,208 times higher than that of the homozygote in the main allele (a/a).

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