ORIGINAL ARTICLE

THE ASSOCIASON OF VITAMIN D RECEPTOR GENE *(VDR)* POLYMORPHISMS WITH HIGH BLOOD PRESSURE IN STROKE PATIENTS OF UKRAINIAN POPULATION

10.36740/WLek202011105

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ABSTRACT

The aim: To study the association of the polymorphisms *VDR* gene with high blood pressure in stroke patients in the Ukrainian population.

Materials and methods: Venous blood of 170 patients with atherothrombotic ischemic stroke (AIS) and 124 healthy individuals (control group) was used for genotyping. Four polymorphisms (*Fok*I, *Bsm*I, *Apa*I, *Taq*I) of gene *VDR* were examined with PCR-RFLP methodology. Statistical analysis was performed by using SPSS-17.0 program.

Results: The correlation of genotypes of polymorphic variants of *FokI*, *BsmI*, *ApaI* and *TaqI* of the *VDR* gene with the development of ischemic atherothrombotic stroke in individuals with normal and high blood pressure was detected. Statistical analysis of the obtained data revealed that among carriers of genotypes F/F, b/b, a/a, a/A, and T/T patients with AI have statistically significantly higher incidence of hypertension than patients in the control group.

Conclusions: It was found that persons with genotypes F/F, b/b, a/a, a/A, and T/T showed a statistically significant relationship between hypertension and the development of IAS. The application of logistic regression has made it possible to establish that the risk of IAS in people with normal blood pressure and genotype F/f is 3.2 times higher than in normotensive homozygotes for the F-allele.

KEY WORDS: high blood pressure, ischemic stroke, gene polymorphism, *VDR*

Wiad Lek. 2020;73(11):2349-2353

INTRODUCTION

Brain lesions are one of the most pressing problems of modern medicine and are among the main factors causing death and disability of the planet's population [1, 2]. Ischemic stroke is a complex disease with wide variations in the age at onset, etiology, comorbidities, and adequacy of collateral circulation. BP is a simple physiologic parameter that is always measured, can be modulated, and may affect the outcome in certain circumstances [3]. All over the world, a lot of attention to a problem of association between allelic gene polymorphism and development of most widespread pathological processes and human diseases has been paid. Recently genes, which depend on the intensity and direction of the calcium-phosphorus metabolism in the body as a whole and in certain tissues, among factors that influence the damage of blood vessels have been named. These includes vitamin D receptor gene (*VDR*). Furthermore, according to clinical trials, there is an inverse correlation between low level of vitamin D and risk factors, such as blood pressure (BP), coronary atherosclerosis and various cardiovascular diseases, arterial hypertension in particular [4, 5].

With the development of molecular genetic technologies, there is many opportunities to study the genetic component of cerebrovascular pathology.

Around the world, attention to the problem of linking allelic gene polymorphism with the development of the most common pathological processes and human diseases is drawn. Among the many polymorphisms studied in these laboratories are genes associated with diseases such as hypertension, coronary heart disease, obesity, acute coronary syndrome, ischemic stroke. One of the genes whose only variants are being studied by scientists is the vitamin D receptor gene. It is likely that there may be a link between vitamin D receptor gene (VDR) polymorphism and acute cerebral circulatory disorders since there are a number of related factors. Vascular wall changes exert its effect through interaction with the vitamin D receptor [6]. The effective activity of this system may depend on many factors, including the polymorphism of the genes encoding the structure of the respective proteins. In turn, VDR encoded by a gene characterized by genetic polymorphism, that is, the existence of different allelic variants of this gene in the population [7]. The most significant VDR gene polymorphisms involved in disease development are *Fok*I, *Bsm*I, *Apa*I, *Taq*I [128, 294]

The lack of data on the association of vitamin D receptor gene polymorphism with ischemic atherotrombotic strokes (IAS) and its developmental factors, namely arterial hypertension not only in Ukrainian but also in other populations is another factor that has prompted our own research.

THE AIM

Aim of our study was the association of the polymorphisms VDR gene with hypertension in stroke patients in the Ukrainian population.

MATERIALS AND METHODS

Subjects. The studied group included 170 patients with IAS (42.4% women and 57.6% men) aged 40 to 85 years (mean age 64.7 ± 0.73 years). The ischemic nature of the stroke according to the anamnesis and clinical picture of the disease, the results of an MRI study of the brain was established. The pathogenic variant of stroke was determined according to TOAST criteria [8], based on the anamnesis and features of the clinical course of the disease, ultrasound Doppler ultrasound, ECG. The control group consisted of 124 patients in whom the absence of cardiovascular pathology was confirmed by anamnestic data collection, electrocardiogram withdrawal and blood pressure measurements. The control group and the group of patients with IAT did not differ in the ratio of persons of different sex (P = 0.294 on the $\chi 2$ criterion), but the mean age of the first (76.7 ± 0.93 years) was significantly higher than the second was (P <0.001) [9].

Amplification and genotyping. DNA for genotyping from the venous blood using commercially available kits (Isogene Lab Ltd, Russia) according to the manufacturer's protocol was extracted. Determination of polymorphisms *Fok*I- (rs 2228570), *Bsm*I- (rs1544410), *Apa*I- (rs7975232), and *Taq*I- (rs731236) of the *VDR* gene using the polymerase chain reaction method followed by restriction fragment length analysis upon detection by agarose gel electrophoresis was performed. Primers synthesized by Metabion (Germany) (Table 1) and enzymes (Taq polymerase and restrictase) by Thermo Scientific (USA) were used. PCR in a GeneAmp PCR thermocycler System 2700 ("Applied Biosystems", USA) was performed.

Detection of the restriction products by horizontal electrophoresis in 2.5% agarose gel (Sigma-Aldrich, USA) was performed containing ethidium bromide (Sigma-Aldrich, USA). The results in ultraviolet rays using an automatic video reading system "Vi-Tran" in a transilluminator ("Biocom" Russia) were visualized [9].

Statistical analysis. Statistical analysis was performed using SPSS-17. Before testing the statistical hypotheses, an analysis of the normality of the distribution of values in the samples was carried out, by determining the asymmetry and excess coefficients using the Willkie-Khan-Shapiro and Lilliefors criteria using the algorithms implemented in SPSS-17. The significance of the differences between the two samples was determined using Student's t test (t). Based on the magnitude of t and the number of degrees of freedom (l = n1 + n2-2), the difference between the two samples (P) was found on the Student's distribution table. The difference was considered significant if the probability of a random difference did not exceed 0.05 (p <0.05). Non-parametric criteria to estimate differences in mean trends and independent samples were used, namely the Fisher exact method for a four-field table (TMF). The use of nonparametric criteria made it possible to find out significant differences in cases where the criterion t did not reveal them.

RESULTS

Analyzing patient groups formed based on genotype *Fok*I polymorphisms on VDR gene (F/F, F/f, f/f), the following data were obtained. Among the carriers of the genotype F/F in the control group was 48.5% of individuals with normal blood pressure and 51.5% of those with hypertension, and in the group of patients with IAS 15% and 85% respectively. Statistical analysis of the data shows that there is a statistically significant correlation between the level of blood pressure and the probability of development of IAS in the homozygote F/F: in patients with arterial hypertension, IAS was found more frequently than in patients with normal blood pressure ($\chi 2 = 9.629$, $P_1 = 0.002$). Among individuals with the F/f genotype, 32.8% of subjects with normal blood pressure and 67.2% of persons with high blood pressure were in control group, and in the group of patients with IAS, their number was 32.8% and 74.7%, respectively. There was no significant difference in the incidence of subjects with normal and increased blood pressure with the F/f genotype in the comparison groups ($\chi 2 = 0.980$, $P_2 = 0.322$). As for carriers' f/f genotype, the control group had 43.3% of those with normal blood pressure and 56.7% with hypertension and among patients with IAS – 33.3% and 66.7% respectively. The frequency of those carriers f/f genotype among people with normal and high blood pressure in the control and experimental group does not get beyond statistical significance ($\chi 2 = 0{,}722$, $P_3 = 0.395$) (Table 2).

Table 1. Conditions for PCR and restriction analysis

SNP	The nucleotide sequence in the primers	Restriction enzime	Restriction fragments, b.p.
*Fok*I rs2228570	Fw 5′-AGCTGGCCCTGGCACTGACTCTG-3′ Rev 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′	*Fok*I	267, 204, 63
*Bsm*I rs1544410	Fw 5′-AGGGAGACGTAGCAAAAGGAG-3′ Rev 5′-TGTCCCCAAGGTCACAATAAC-3′	*Bsm*I	425, 232, 193
*Apa*I rs7975232	Fw 5′-CAGAGCATGGACAGGGAGCAA-3′ Rev 5′-CACTTCGAGCACAAGGGGCGTTAGC-3′	*Apa*I	501, 284, 217
*Taq*I rs731236	Fw 5′-CAGAGCATGGACAGGGAGCAA-3′ Rev 5′-CACTTCGAGCACAAGGGGCGTTAGC-3′	*Taq*I	501, 294, 207

Table 2. Distribution of individuals of different genotypes for VDR gene polymorphisms in the control group and the group of patients with IAS depending on the size of the arterial pressure

SNP	Genotype	Arterial hypertension	Ischemic stroke n (%)	Control group n (%)	P
*Fok*I	F/F	–	6 (15.0)	16 (48.5)	0.002 (1)
		+	34 (85.0)	17 (51.5)	
	F/f	–	23 (25.3)	19 (32.8)	0.322 (2)
		+	68 (74.7)	39 (67.2)	
	f/f	–	13 (33.3)	13 (43.3)	0.395 (3)
		+	26 (66.7)	17 (56.7)	
*Bsm*I	b/b	–	16 (22.5)	23 (41.1)	0.025 (4)
		+	55 (77.5)	33 (58.9)	
	b/B	–	19 (25.7)	19 (37.3)	0.167 (5)
		+	55 (74.3)	32 (62.7)	
	B/B	–	7 (28.0)	6 (42.9)	0.345 (6)
		+	18 (72.0)	8 (57.1)	
*Apa*I	a/a	–	7 (15.6)	17 (43.6)	0.005 (7)
		+	38 (84.4)	22 (56.4)	
	a/A	–	22 (25.9)	21 (42.0)	0.052 (8)
		+	63 (74.1)	29 (58.0)	
	A/A	–	13 (32.5)	10 (31.2)	0.910 (9)
		+	27 (67.5)	22 (68.8)	
*Taq*I	T/T	–	15 (22.1)	21 (39.6)	0.036 (10)
		+	53 (77.9)	32 (60.4)	
	T/t	–	22 (26.8)	21 (38.9)	0.139 (11)
		+	60 (73.2)	33 (61.6)	
	t/t	–	5 (25.0)	6 (42.9)	0.273 (12)
		+	15 (75.0)	8 (57.1)	

Table 3. Risk analysis IAS depending on genotype FokI polymorphism of VDR gene in patients with normal and high blood pressure (logistic regression method)

	Genotype	CR	SE	WS	P	OR	95% CI for OR lower	95% CI for OR upper
Normal BP	F/f	1.172	0.570	4.222	0.040	3.228	1.056	9.872
	f/f	0.981	0.619	2.512	0.113	2.667	0.793	8.969
Arterial hypertension	F/f	0.137	0.359	0.146	0.702	0.872	0.432	1.761
	f/f	0.268	0.431	0.388	0.533	0.765	0.329	1.779

Note: Homozygotes for f-allele (f/f) are compared with carriers of F-allele (F/f+F/F). CR – regression coefficient, SE – standard error, WS – Wald statistics, P – statistical significance, OR – risk ratio, CI – confidence interval.

Application of logistic regression method made it possible to establish that the risk of occurrence of IAS in persons with normal blood pressure and genotype F/f is 3.2 times higher than in normotensive homozygotes according to F-allele (Table 3).

The study of the frequencies of persons with normal and high blood pressure in the comparison groups depending on the variants of the genotype according to the *Bsm*I-polymorphism of the *VDR* gene was informative. Thus, among the carriers of the b/b genotype, 41.1% of persons with normal pressure and 58.9% with high blood pressure in the control group and 22.5% and 77.5% in the group of patients with IAS were detected respectively. Statistical analysis of the data revealed that among carriers of the b/b genotype, patients with IAT have a significantly higher frequency of hypertension than patients in the control group ($\chi 2$ = 5,055, P_4 = 0.025). Among persons with the b/B genotype, 37.3% of those with normal pressure and 62.7% with high blood pressure were in control, and in the group of patients with IAS, their number was 25.7% and 74.3%, respectively. There are no differences in the frequencies of individuals according to this genotype in the comparison groups

($\chi2$ = 1.913, P_5 = 0.167). Regarding carriers of genotype B/B, 42.9% of subjects with normal pressure and 57.1% with hypertension in the control group and among patients, there were 28.0% and 72.0% were found respectively. Frequency of carriers of genotype B/B among representatives with different values of blood pressure in the control and experimental groups does not get beyond statistical significance ($\chi2$ = 0,891, P_6 = 0,345) (Table 2). Therefore, in homozygotes for the b-allele and not the B-allele carriers, patients with IAT have a higher incidence of hypertension than patients in the control group.

In the analysis of groups of patients formed based on the genotype by the *Apa*I polymorphism of the VDR gene (a/a, a/A, A/A), the following data were obtained. Among the carriers of the genotype a/a in the control group, there were 43.6% of persons with normal and 56.4% of persons with high blood pressure, and in the group of patients with IAS 15.6% and 84.4% respectively. Statistical analysis of the data indicates that homozygotes a/a have a statistically significant relationship between the level of blood pressure and the likelihood of developing IAS: in individuals with arterial hypertension IAS was found more often than in patients with normal blood pressure ($\chi2$ = 8,046, P_7 = 0,005). There were 42.0% of subjects with normal BP and 58.0% with increased BP among control group with the A/A genotype, and in the IAS group, their numbers were 25.9% and 74.1%, respectively. Therefore, the incidence of subjects with normal and increased blood pressure with a/A genotype in the comparison groups is statistically significant. ($\chi2$ = 3.768, P_8 = 0.052). Regarding A/A genotype carriers, 31.3% of subjects with normal blood pressure and 68.8% with hypertension was in the control group, and among patients with IAS 32.5% and 67.5% were found respectively. The frequency of A/A genotype carriers among patients with normal and elevated blood pressure in the control and study group was not statistically significant ($\chi2$=0.013, P_9 = 0.910) (Table 2).

Study of frequencies of persons with normal and high blood pressure in comparison groups depending on variants of the genotype by *Taq*I-polymorphism of the VDR gene was informative too. Thus, among the carriers of the T/T genotype, 39.6% of persons with normal pressure and 60.4% with high blood pressure were detected in the control group, and 22.1% and 77.9% respectively in the group of patients with IAS. Statistical analysis of the data revealed that among carriers of the T/T genotype, patients with IAS have a significantly higher incidence of hypertension than patients in the control group ($\chi2$ = 4.396, P_{10} = 0.036). Among those with the T/t genotype, 38.9% had normal pressure and 61.1% had increased blood pressure in the control group, and 26.8% and 73.2% in the IAS group respectively. There were no differences in the frequencies of individuals according to this genotype in the comparison groups ($\chi2$ = 2,190, P_{11} = 0,139). As for carriers of t/t genotype, in the control group 42.9% of those with normal blood pressure and 57.1% with hypertension and among these patients with IAS 25.0% and 75.0 % were found respectively. The frequency of carriers of the genotype t/t among representatives with different values of blood pressure in the control and experimental group does not go beyond statistical significance ($\chi2$ = 1,200, P_{12} = 0,273) (Table 2). Therefore, in T-allele homozygotes and not in the t-allele carriers, patients with IAS have a higher incidence of hypertension than patients of the control group.

DISCUSSION

Worldwide, ischemic stroke accounts for 85-90% of all strokes, of which 60% is associated with the development of hypertension, diabetes, heart disease, high cholesterol, obesity and smoking. Another 40% of atherothrombotic ischemic stroke, about half are associated with other acquired pathologies and the other half is caused by unknown factors [10]. Determining the role of a particular gene in the development of ischemic stroke is challenging. This is due to its interaction with other genes and factors, as well as with related diseases [11]. Thus, there is an increase in the risk of disease was associated with the carrier of one gene in combination with other genes whose actions are synergistic with respect to the risk of ischemic stroke [11, 12]. It is important to note that there is a genetic heterogeneity of ischemic strokes or, in other words, each clinical and pathogenic variant of a stroke has unique gene combinations. The risk of ischemic stroke increases not only under the influence of polymorphism of one gene, but under also when alleles of several genes are combined, that is, there is a polygenic hereditary predisposition to thrombotic lesions of brain vessels is established [12].

VDR exercises its influence on the development of hypertension using different mechanisms. However, the most important of these is influenced by VDR on the process of calcification of the vascular wall through the regulation of Matrix Gla-Protein synthesis [13-15].

In our study, the assosiation between the *Fok*I-, *Bsm*I-, *Apa*I-, and *Taq*I-polymorphisms of the *VDR* gene with the development of IAS in individuals with normal and increased blood pressure have been identified. When analyzing groups of patients formed with the FokI polymorphism genotype among carriers of the F/F genotype, there is a statistically significant relationship between the level of the blood pressure and the probability of IAS development [16-18].

The data we obtained are consistent with those of other sources [19]. In patients with hypertension with the genotypes F / F, F / f, compared with patients with the genotype f / f, were recorded higher values of daily average AP_{syst} and average AP_{syst} per day was reported.

Shuai Lu, et al. informed that the Fok1 polymorphism may play a protective role in Coronary artery disease (CAD), and the possible protective role in Apa1 CA genotype in CAD patients with T2DM needs further studies. The Taq1 polymorphism is found to be associated with a significant increase in CAD risk based on our analysis; moreover, increased risk in Apa1 polymorphism in CAD patients without T2DM and Bsm1 polymorphism in Caucasian group is also detected [20].

CONCLUSIONS

It was found that persons with genotypes F/F, b/b, a/a, a/A, and T/T showed a statistically significant relationship between hypertension and the development of IAS. The application of logistic regression has made it possible to establish that the risk of IAS in people with normal blood pressure and genotype F/f is 3.2 times higher than in normotensive homozygotes for the F-allele.

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Conflict of interest:

The Authors declare no conflict of interest.

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Received: 28.02.2020
Accepted: 08.07.2020

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article