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Evaluation of VDR and PAI allelic genes in patients with avascular necrosis of the femoral head

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Objective To evaluate the informative value of the carrier status for allelic variations that determine the sensitivity of tissues to calcitriol (*VDR*) and are involved in familial thrombophilia and hypofibrinolysis (*PAI-1*) as molecular genetic markers of avascular necrosis of the femoral head (AVNFH). **Material and methods** A clinical and laboratory study of 300 AVNFH patients, residents of European Russia, was carried out. A comparative analysis of the alleles and genotypes frequency distribution of polymorphisms rs11568820 and rs1544410 of the *VDR* gene, as well as rs1799889 of the *PAI-1* gene in AVNFH patients was performed. **Results** AVNFH patients showed a significant increase in the frequencies of the G/G genotype (P = 3.0E-9) and the G allele (P = 0.05) of the rs11568820 *VDR* polymorphism (P = 2.10E-08) as compared to controls. The frequency of the A/A genotype of the rs1544410 *VDR* locus in AVNFH individuals was higher than that in controls (P = 0.05). **Discussion** Carriers of the genotype G / G A-3731G (Cdx2) of the *VDR* gene were shown to have a 2.1-fold increased risk of developing AVNFH; carriers of the G allele appeared to have a 2.3-fold increased risk of AVNFH. The findings showed that the carriership of the 5G allele of the polymorphic locus *PAI-1* -675 4G > 5G (rs1799889) is detected 1.4 times more often in AVNFH patients than in individuals from the population sample. The risk of developing the pathology is increased 2 times with the carriership of the 5G/5G genotype of this polymorphic locus. **Conclusion** Carriers of genotypes G/G rs11568820 *VDR* (allele G), A/A rs1544410 *VDR* and 5G/5G (allele 5G) at the polymorphic locus rs1799889 *PAI-1* have an increased risk of developing AVNFH. This allows the use of the molecular genetic markers in the early diagnosis of AVNFH in individuals who are at greater risk for the disease.

Keywords: avascular necrosis, femoral head, allele variant, *VDR*, *PAI*

INTRODUCTION

A vascular necrosis of the femoral head (AVNFH) is a severe degenerative disease resulting from bone destruction, impaired microcirculation, and bone marrow adipose dystrophy [1–3]. These factors affect the stress bone remodeling. Etiologically AVNFH can be classified into two types: primary/idiopathic and secondary. Secondary AVNFH can be diagnosed in cases of bone or bone marrow microcirculation dysfunction being associated with an identifiable cause, such as traumatic injuries, systemic administration of steroids or bisphosphonates, excessive alcohol consumption, sickle cell anemia, autoimmune diseases, chemotherapy or malignancies [4-6]. The cause of primary or idiopathic AVNFH is unknown. Genetically determined target organ and tissue sensitivity to calcitriol, hereditary thrombophilia and hypofibrinolysis are described in the medical literature as potential causes of idiopathic types of AVNFH [7–9]. Calcitriol is a physiologically active terminal metabolite of vitamin D produced in the skin under the influence of ultraviolet irradiation (cholecalciferol) and derived from food (ergocalciferol). Human mineral metabolism depends not only on calcitriol concentraton but also on the target organ sensitivity [10]. The intracellular calcitriol receptor expressed by the vitamin D receptor (*VDR*) gene determines target organ and tissue sensitivity to calcitriol at the final stage of D-vitamin metabolism [11]. AVNFH is a multifactorial disease and allelic variants of the *VDR* gene can be a risk factor [12–14]. Genetic mutations of proteins involved in coagulation and fibrinolysis may have a role in the pathogenesis of avascular necrosis. Plasminogen activator inhibitor-1 (*PAI-1*) is one of the main components of the thrombolytic plasminogen-plasmin system of fibrinolysis [15]. Homozygous 4G/4G variant of the plasminogen activator inhibitor gene -675 5G > 4G (rs1799889) *PAI-1* was reported to be associated with a greater risk of AVNFH [16-18]. The study of gene polymorphism that determines target organ and tissue sensitivity to calcitriol, hereditary thrombophilia and hypofibrinolysis, as molecular genetic markers of the AVNFH is important to be used as identification tool.

The objective of the study was to evaluate the informative value of the carrier status for allelic variations that determine target organ and tissue sensitivity to calcitriol (*VDR*), hereditary thrombophilia and hypofibrinolysis (*PAI-1*) as molecular genetic markers of AVNFH.

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MATERIAL AND METHODS

The frequency of significant allelic variants A-3731G (Cdx2) and +283 G > A (BsmI) of the vitamin D receptor *VDR* gene and -675 5G > 4G (rs1799889) of the plasminogen activator inhibitor PAI-1 gene was reviewed in patients with a verified diagnosis of AVNFH (the main group) and compared to the population controls including the reported data on healthy Russian individuals of both sexes. 300 AVNFH patients (168 females and 132 males) who sought specialized medical care were examined at LLC HuangDi AVNFH Medical Center. The average age was 56.5 ± 0.1 years for males and 59.1 ± 0.1 years for females. The group of the examinees was ethnogenetically homogeneous and included residents of European Russia. The patients were diagnosed with AVNFH based on the results of physical examination, instrumentation and laboratory findings, and anamnestic data evaluating dysplastic changes in the joints, physical overload, stress, injuries, low-energy fractures sustained by the patient or parents, excessive alcohol consumption, smoking, glucocorticoid therapy, the presence of systemic diseases, blood diseases, indigestible dairy products; the postmenopausal duration in women and symptoms related to low testosterone levels in men.

Hip joints were assessed with computer tomography and magnetic resonance imaging and conventional radiography in 3 projections with images taken in a supine position, on the abdomen, and the Löwenstein view. The AVNFH was staged with the the Ficat and the Association Research Circulation Osseous (ARCO) classifications. The Harris Hip score was used to assess hip function. BeamMed Sunlight MiniOmni Bone Densitometer (Israel) was used to measure the mineral density and bone quality of the proximal phalanx of the 3d finger of the non-dominant hand and the radius. The Thermo Scíentífic KingFisher Flex hardware was used for automated extraction of DNA from patients' venous blood leukocytes. Single nucleotide polymorphisms of the *VDR* and *PAI-1* genes were detected in PCR and pyrosequences methods using an Eppendorf MasterCycler Nexus Gradient amplifier (Germany) and equipment with Pyro Mark Q24 QIAGEN software (Germany) at a private diagnostic laboratory. Polymerase and buffer solution supplied by Isogen Laboratory LLC were used for PCR; allele-specific oligonucleotides were supplied by Eurogen. The findings reported by population series produced in European Russia were used for comparisons [11, 19, 20].

Statistical analysis included checking for compliance of the samples of the examinees and controls with the Hardy-Weinberg equilibrium, comparing the frequencies of the polymorphisms between the groups. The exact Fisher test (F-test) and Pearson's χ^2 test were used to identify significant differences with the WinPepi software package (http://www.brixtonhealth.com/pepi4windows.html). Associations between the disease and the genotype were determined with the use of multiplicative and additive models of inheritance. The odds ratio [21] (odds ratio – OR) with a 95 % confidence interval (95 % CI) was calculated with significant differences in the variables of AVNFH patients and controls detected with software "Calculator for statistics in case – control studies" (https://calc.pcr24.ru/index.php).

RESULTS AND DISCUSSION

The distribution of genotype frequencies across the loci explored in the control samples and groups of patients corresponded to the theoretically expected Hardy-Weinberg equilibrium ($p > 0.05$), with the exception of two cases: rs11568820 (control) $P = 0.017$; rs1544410 $P = 0.052$. This was taken into account to complete data analysis reports. The human *VDR* vitamin D receptor gene is localized on chromosome 12q12-q14. About 30 single nucleotide polymorphisms have been found in the VDR gene and can be identified by appropriate restriction endonuclease enzymes. The best known are ApaI (rs7975232), BsmI (rs1544410), FokI (rs2228570), TaqI (rs731236) and Cdx2 (rs11568820) [22, 23]. The A-3731G (Cdx2) polymorphism (rs 11568820) is located in the 5'-promoter region of the VDR gene. In carriers of the G (Cdx2) allele, transcriptional activity of VDR gene is reduced to 70 % of the level of carriers of allele A. The studies of the polymorphism report the presence of the mutant A allele providing the carrier's resistance to loss of bone mineral density with reduced calcium intake [19, 24]. BsmI polymorphism is localized in the 3'-regulatory region and is involved in the regulation of mRNA stability. The AA genotype is associated with an increased risk of osteoporosis in postmenopausal women and a response to antiresorptive therapy [25, 26], and a higher risk of femoral fracture as compared to the GG genotype [27].

Review of the findings (Table 1) showed significant differences in the frequencies of carriers of the genotypes GG, AG and AA of the polymorphic

locus A-3731G (Cdx2) of the VDR gene (rs11568820) in AVNFH patients and controls measuring 71.0, 26.0, 3.0 % and 44.8, 48.8 and 6.4 %, respectively (χ^2 = 35.26; df [0, 1, 2], 1, P = 3.0 E-9). AVNFH was likely to increase threefold (OR = 3.02: CI 2.12–4.29) with the carrier of G/G genotype of the A-3731G (Cdx2) locus of the *VDR* gene. The percentage of carriers of the G allele was 84 % in AVNFH patients and significantly exceeded that of the control group (69.2 %) (F = 0.000041, ξ^2 =17.01). AVNFH was likely to increase 2.3-fold (OR = 2.34, CI 1.55-3.52) with the carrier G allele. There were no statistically significant differences in allele frequencies in the +283 A > G polymorphic locus (BsmI) of the *VDR* gene in AVNFH group and controls (Table 1). There was a slightly impaired Hardy-Weinberg equilibrium at the locus (P = 0.052). There were significant differences in the genotype frequencies at the locus detected in AVNFH and controls measuring 51.0, 41.7, 7.3 % and 43.3, 38.3, 18.4 %, respectively (χ^2 = 3.85; df [0, 1, 2] 1, P = 0.05) (Table. 1). The risk of developing the pathology increased with carrier of the A/A genotype of the VDR gene (OR = 2.36; 95 % CI: 1.03–5.42).

The *PAI-1* gene encodes an inhibitor of the plasminogen activator type 1 (*PAI-1*), one of the main inhibitors of fibrinolysis. The *PAI-1* gene is located on human chromosome 7q21. 3-22 [28, 29]. The main PAI-1 polymorphism is a single insertion/deletion of guanine (G) at position 675 in the *PAI-1* promoter region and can cause changes in the gene transcription rate. The polymorphism includes two alleles that contain four or five consecutive guanine bases (4G and 5G). Individuals being homozygous for the 4G allele (4G/4G) have higher gene transcription levels and a higher plasma concentration of *PAI-1* as compared to homozygotes for the 5G allele (5G/5G) and therefore may have a higher risk of intravascular thrombosis. The plasma levels of *PAI-1* appear to be intermediate in heterozygotes (4G/5G). The 4G allele has higher transcriptional activity (six-time mRNA production) than 5G, and is characterized by increased *PAI-1* expression. An increase in the concentration of *PAI-1* in plasma leads to an increase in thrombosis. The findings reported in meta-analyses [30, 31] showed that *PAI-1* -675 4G > 5G (rs1799889) polymorphism may be associated with the risk of AVNFH.

The data presented in Table 2 showed that the 5G allele of the *PAI-1* -675 4G > 5G polymorphic locus (rs1799889) was more common for AVNFH patients than for individuals of the population sample: 50.0 % vs. 41.95 % (OR = 1.39; 95 % CI:1.04–1.85). There were significant differences in the genotype frequencies at the locus detected in the population sample and AVNFH patients measuring 51.0, 41.7, 7.3 % and 43.3, 38.3, 18.4 %, respectively (χ^2 = 4.64; df [0, 1, 2], 1, P = 0.03) (Table 2). The risk of AVNFH was increased 2 times with carrier of the 5G/5G genotype at the locus of the *PAI-1* gene (OR = 1.98; 95 % CI: 1.22–3.20).

Table 1

Frequency of alleles and genotypes of polymorphic loci of the *VDR* gene in AVNFH patients and healthy individuals

Groups and number of individuals (n)	A-3731G (Cdx2) (rs11568820)						
	Frequency of alleles, %			Frequency of genotypes, %			
	G	A	F-test	GG	AG	AA	χ^2; [0, 1, 2], df = 1
Population sample (250)*	69.2	30.8	0.000041	44.8	48.8	6.4	35.26
AVNFH patients (300)	84.0	16.0	ξ^2 = 17.01	71.0	26.0	3.0	P = 3.0 E-9
Population sample (96)**	+283 A > G (BsmI) (rs1544410)						
	G	A	F-test	GG	AG	AA	χ^2; [0, 1, 2], df = 1
	71.9	26.1	0.044288	51.0	41.7	7.3	3.85
AVNFH patients (300)	63.8	36.2	ξ^2 = 4.31	43.3	41.0	15.7	P = 0.05

Note: * as reported [19];** as reported [11]

Table 2

Frequency of alleles and genotypes of the -675 5G>4G locus of the *PAI-1* gene in AVNFH patients and healthy individuals

Groups and number of individuals (n)	-675 5G>4G (rs1799889)						
	Frequency of alleles, %			Frequency of genotypes, %			
	5G	4G	χ^2; df = 1	5G/5G	5G/4G	4G/4G	χ^2; [0, 1, 2], df = 1
Population sample (228)*	41.95	58.05	5.03	17.5	48.7	33.8	4.64
AVNFH patients (162)	50.0	50.0	P = 0.02	29.7	40.7	29.6	P = 0.03

Note: * as reported [10]

CONCLUSION

The findings showed that individuals carrying the G/G genotype of the A-3731G (Cdx2) locus of the *VDR* gene showed threefold likelihood of developing AVNFH (OR = 3.02: CI 2.12 – 4.29). The proportion of carriers of the G allele among individuals with the pathology (84 %) significantly exceeded that of controls (69.2 %) (F = 0.000041, $\xi^2 = 17.01$). The G allele was associated with a 2.3-fold increase in the probability of developing AVNFH (OR = 2.34, CI 1.55-3.52).

There were no statistically significant differences in allele frequencies in the +283 A > G polymorphic locus (BsmI) of the *VDR* gene in AVNFH patients and controls. However, there were statistically significant differences in the frequency of genotypes of the locus in AVNFH patients and individuals from the population sample measuring 51.0, 41.7, 7.3 % and 43.3, 38.3, 18.4 %, respectively ($\chi^2 = 3.85$; df [0, 1, 2], 1, P = 0.05). There was a greater risk of developing AVNFH in individuals carrying the G/G genotype in the *VDR* gene (OR = 2.36; 95 % CI: 1.03–5.42).

The findings showed that the carrier of the 5G allele of the polymorphic locus *PAI-1* -675 4G > 5G (rs1799889) was more common for AVNFH patients than for individuals from the population sample: 50.0 % vs. 41.95 % (OR = 1.39; 95 % CI: 1.04-1.85). However, there were statistically significant differences in the frequency of genotypes of the locus in AVNFH patients and controls measuring 51.0, 41.7, 7.3% and 43.3, 38.3, 18.4 %, respectively ($\chi^2 = 4.64$; df [0, 1, 2], 1, P = 0.03). There was a twofold increase in the risk of AVNFH in individuals carrying the PAI-1 gene of the 5G/5G genotype at the locus (OR = 1.98; 95 % CI: 1.22–3.20).

Therefore, the findings indicated to the possibility to trace out the hereditary predisposition to AVNFH in patients with musculoskeletal diseases and, consequently, provide timely prevention of the disease.

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