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

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INTRODUCTION

A vascular necrosis of the femoral head (AVNFH) is a severe degenerative disease resulting from bone destruction, impaired microrcirculation, 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 concentration 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.
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 systemic diseases, blood diseases, indigestible dairy products, 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 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 Scientific 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 $\chi^2$ 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) $P = 0.017$; rs15444410 $P = 0.052$. 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 Apal (rs7975232), BsmI (rs15444410), 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 ($\chi^2 = 35.26$; df [0, 1, 2], $P = 3.0 \times 10^{-9}$). 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 %) ($F = 0.000041$, $\xi^2 = 17.01$). 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 P4I-1 gene encodes an inhibitor of the plasminogen activator type 1 (P4I-1), one of the main inhibitors of fibrinolysis. The P4I-1 gene is located on human chromosome 7q21.3-22 [28, 29]. The main P4I-1 polymorphism is a single insertion/deletion of guanine (G) at position 675 in the P4I-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 P4I-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 P4I-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 P4I-1 expression. An increase in the concentration of P4I-1 in plasma leads to an increase in thrombosis. The findings reported in meta-analyses [30, 31] showed that P4I-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 P4I-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 ($\chi^2 = 3.85$; df [0, 1, 2], $P = 0.05$) (Table 2). The risk of AVNFH was increased 2 times with carrier of the 5G/5G genotype at the locus of the P4I-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) | +283 A > G (BsmI) (rs1544410) |
|-------------------------------------|-----------------------------|---------------------------------|
|                                     | Frequency of alleles, %      | Frequency of genotypes, %       |
|                                     | G   | A   | F-test | GG  | AG  | AA  | $\chi^2; [0, 1, 2]$, 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 | $\xi^2 = 17.01$ | 71.0 | 26.0 | 3.0 | P = 3.0 E-9 |

| Groups and number of individuals (n) | -675 5G>4G (rs1799889) |
|-------------------------------------|-------------------------|
|                                     | Frequency of alleles, %  | Frequency of genotypes, % |
|                                     | 5G | 4G | $\chi^2; df = 1$ | 5G/5G | 5G/4G | 4G/4G | $\chi^2; [0, 1, 2]$, 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 [19]; ** as reported [11]
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, χ^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 (χ^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-I -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 (χ^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.

REFERENCES

1. Banerjee S., Kapadia V.N., Jauregui J.J., Cherian J.J., Mont M.A. Natural History of Osteonecrosis. Osteonecrosis. Koo K.H., Mont M.A., Jones L.C., eds. 2014. Berlin, Heidelberg, Springer, 2014, pp. 161-164. DOI: 10.1007/978-3-642-35767-1_20
2. Volkov E.E., Ketsin Khan. Asepticheskii nekroz golovki bedrennoi kosti. Bezoperatsionnoe lechenie: monografija [Aseptic necrosis of the femoral head. Non-surgical treatment: monograph]. M., Peri-Press, 2010, 110 p. (in Russian)
3. Seamon J., Keller T., Saleh J., Cui Q. The pathogenesis of nontraumatic osteonecrosis. Arthritis, 2012, vol. 2012, pp. 601763. DOI: 10.1155/2012/601763
4. Ilinykh E.V., Barskova V.G., Lidov P.I., Nasonov E.L. Osteonekroz. Chast 1. Faktory riska i patogenez [Osteonecrosis. Part 1. Risk factors and pathogenesis]. Sovremennaia Revmatologija, 2013, vol. 7, no. 1, pp. 17-24. (in Russian)
5. Li Z., Huang C., Yang B., Hu W., Chan M.T., Wu W.K.K. Emerging roles of long non-coding RNAs in osteonecrosis of the femoral head. Am. J. Transl. Res., 2020, vol. 12, no. 9, pp. 5984-5991.
6. Haydock M.M., Elhadamani S., Alshariedi M. Long-term directorial anticoagulation in primary osteonecrosis with elevated plasminogen activation inhibitor. SAGE Open Med. Case Rep., 2019, vol. 7, 2050313X19827747. DOI: 10.1177/2050313X19827747
7. Orth P., Anagnostakos T. Coagulation abnormalities in osteonecrosis and bone marrow edema syndrome. Orthopedics, 2013, vol. 36, no. 4, pp. 290-300. DOI: 10.3928/01477447-20130327-08
8. Seamon J., Keller T., Saleh J., Cui Q. The pathogenesis of nontraumatic osteonecrosis. Arthritis, 2012, vol. 2012, pp. 601763. DOI: 10.1155/2012/601763
9. Hadjigeorgiou G., Dardiotis E., Dardioti M., Karantanas A., Dimitroulias A., Malizos K. Genetic association studies in osteonecrosis of the femoral head. BMJ Open, 2013, vol. 3, no. 3, pp. e001763.
10. Holick M.F. Vitamin D deficiency. N. Engl. J. Med., 2007, vol. 357, no. 3, pp. 266-281. DOI: 10.1056/NEJMra070553
11. Koo K.H., Osteonecrosis. Arthritis, 2012, vol. 2012, 601763.
12. Kozev A.I., Vershubskaia G.G., Negusheva M.A. Polimorfizm gena reseptora vitamina D (VDR) v vyborkakh naseleniia Evropeiskoi Rossii i Priurialia [Vitamin D receptor (VDR) gene polymorphism in samples of the population of European Russia and the Urals]. Permskii Meditsinskii Zhurnal, 2016, vol. 33, no. 5, pp. 60-66. (in Russian)
13. Zeng Z., Wang B., Pan H. Relation between osteonecrosis of the femoral head and PAI-1 4G/5G gene polymorphism: a meta-analysis. Int. J. Clin. Exp. Med., 2015, vol. 8, no. 11, pp. 20337-20342.
14. Volkov E.E., Gordeev M.V., Goloshchapov A.P., Romanova A.R., Nostaeva S.E. Issledovanie polimorfnykh lokusov genov CALCR, COL1A1, VDR, LCT u patsientov s asepticheskim nekrozom golovki bedrennoi kosti [Study of polymorphic loci of CALCR, COL1A1, VDR, LCT in patients with aseptic necrosis of the femoral head]. Genij Ortopedii, 2018, vol. 24, no. 3, pp. 335-340.
15. Pizova N.V. Trombofilii: geneticheskie polimorfizmy i sosudistye katastrofy [Thrombophilias: genetic polymorphisms and vascular accidents]. M., IMA-PRESS, 2013, 248-PAGES. (in Russian)
16. Kim H., Cho C., Cho Y., Yoon K., Kim S. Significant associations of PAI-1 genetic polymorphisms with osteonecrosis of the femoral head. BMC Musculoskelet. Disord., 2011, vol. 12, pp. 160. DOI:10.1186/1471-2474-12-160
17. Lu Y., Yu Q., Guo W., Hao Y., Sun W., Cheng L. Effect of glucocorticoids on the function of microvascular endothelial cells in the human femoral head bone. Adv. Clin. Exp. Med., 2020, vol. 29, no. 3, pp. 345-353. DOI: 10.17219/acem/112602
18. Li Y., Liu F.X., Yuan C., Meng L. Association between plasminogen activator gene polymorphisms and osteonecrosis of the femoral head susceptibility: A case-control study. Medicine (Baltimore), 2017, vol. 96, no. 42, pp. e7047. DOI:10.1097/MD.0000000000007047
19. Bazilevskaia E.M., Iakubova I.Sh., Topanova A.A. Otsenka geneticheskoi predraspolozhennosti molodykh zhitelei g. Sankt-Peterburga k zabolevaniam, sviazannym s narusheniem obmena kaltsiia [Assessment of genetic predisposition of young residents of St. Petersburg to diseases associated with impaired calcium metabolism]. Profilakticheskie i Klinicheskie Meditsina, 2014, no. 3 (52), pp. 96-101. (in Russian)
20. Shikhbabaeva D.I., Polushkina L.B., Shuvaev V.A., Martynevich I.S., Kapustin S.I., Zamotina T.B., Fominykh M.S., Udalova V.I., Zotova I.I., Shmeleva V.M., Smirnova O.A., Voloshin S.V., Bessmel'tsev S.S., Chechetkin A.V., Abdulkadyrov K.M. Genetic markers of hereditary thrombophilia and the risk of thrombotic complications in patients with polycythemia vera. *Klinicheskaia Onkogematologiia. Fundamentalnye Issledovaniia i Klinicheskaia Praktika*, 2017, vol. 10, no. 1, pp. 85-92. (in Russian)

21. Moskalenko M.V., Aseev M.V., Kotova S.M., Baranov V.S. Analysis of the association of alleles of CollA1, VDR and CALC1 genes with osteoporosis development. *Ekologicheskaia Genetika*, 2004, vol. 2, no. 1, pp. 38-43. (in Russian)

22. Uitterlinden A.G., Fang Y., Van Meurs J.B., Pols H.A., Van Leeuwen J.P. Genetics and biology of vitamin D receptor polymorphisms. Gene, 2004, vol. 338, no. 2, pp. 143-156. DOI:10.1016/j.gene.2004.05.014

23. Palshina A.M., Palshina S.G., Safonova S.L., Palshin V.G. On the genetic markers of hereditary thrombophilia and the risk of thrombotic complications in patients with polycythemia vera. *Geneticheskie markery nasledstvennoi trombofilii i risk tromboticheskikh oslozhnenii u bolnykh s istinnoi politsitemiei*. Klinicheskaia Onkogematologiia. Fundamentalnye Issledovaniia i Klinicheskaia Praktika, 2017, vol. 10, no. 1, pp. 85-92. (in Russian)

24. Palshina A.M., Palshina S.G., Safonova S.L., Palshin V.G. Na zametku klinitsistu: sovremennyi vzgliad na metabolizm vitamina D i polimorfizm gena reseptora vitamina D [Notes to the clinician: Modern view of vitamin D metabolism and vitamin D receptor gene]. *Vestnik Severo-vostochnogo Federalnogo Universiteta im. M.K. Ammosova. Seriia: Meditsinskie nauki*, 2018, no. 3 (12), pp. 34-42. (in Russian) DOI: 10.25587/SVFU.2018.16.7.1256

25. Marozik P.M., Tamulaite M., Rudenka E., Alekna V., Mosse I., Rudenka A., Samokhovec V., Kobets K. Association of Vitamin D Receptor Gene Variation with Osteoporosis Risk in Belarusian and Lithuanian Postmenopausal Women. *Front. Endocrinol. (Lausanne)*, 2018, vol. 9, pp. 305. DOI: 10.3389/fendo.2018.00305

26. Zhang L., Yin X., Wang J., Xu D., Wang Y., Yang J., Tao Y., Zhang S., Feng X., Yan C. Associations between VDR Gene Polymorphisms and Osteoporosis Risk and Bone Mineral Density in Postmenopausal Women: A systematic review and Meta-Analysis. *Sci. Rep.*, 2018, vol. 8, no. 1, pp. 981. DOI: 10.1038/s41598-017-18670-7

27. Horst-Sikorska W., Dytfeld J., Wawrzyniak A., Marcinkowska M., Michalak M., Franek E., Napiórkowska L., Drwęska N., Słomski R. Vitamin D receptor gene polymorphisms, bone mineral density and fractures in postmenopausal women with osteoporosis. *Mol. Biol. Rep.*, 2013, vol. 40, no. 1, pp. 383-390. DOI: 10.1007/s11033-012-2072-3

28. Klinger K.W., Winquist R., Riccio A., Andreasen P.A., Sartorio R., Nielsen L.S., Stuart N., Stanislovitis P., Watkins P., Douglas R., Grzeschik K.H., Alitako K., Blasi F., Dano K. Plasminogen activator inhibitor type 1 gene is located at region q21.3-q22 of chromosome 7 and genetically linked with cystic fibrosis. *Proc. Natl. Acad. Sci. USA*, 1987, vol. 84, no. 23, pp. 8548-8552. DOI: 10.1073/pnas.84.23.8548

29. Trifonova E.A., Gabdulina T.V., Bukharina I.Iu., Stepanov V.A. Plasminogen activator type 1 4G/5G polymorphism contributes to osteonecrosis of the femoral head susceptibility: Evidence from a Systematic Review and Meta-analysis. *Arch. Bone Jt. Surg.*, 2018, vol. 6, no. 6, pp. 468-477. DOI:10.22038/ABJS.2018.31668.1828

30. Yang J., Jing M., Yang X. Association between genetic polymorphisms and osteonecrosis in steroid treatment populations: a detailed stratified and dose-response meta-analysis. *Biosci. Rep.*, 2019, vol. 39, no. 5. BSR20190024. DOI: 10.1042/BSR20190024

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