Association of VDR gene polymorphisms with the risk of osteoporosis in Uzbekistan

Accepted 15th January, 2019

ABSTRACT

A direct search was conducted for new polymorphisms in the second exon of the vitamin D receptor (VDR) gene in osteoporosis. In this study, an attempt was made to search for new polymorphisms in the fragment (12q13.11; 47879122-47878872) of the VDR gene among representatives of the Uzbek population. Sequencing of the fragment (12q13.11; 47879122-47878872) of the VDR gene locus of 57 DNA samples (42 patients with osteoporosis and 15 healthy individuals) showed the presence of two (2) polymorphisms: rs2228570 and rs2228572. The obtained results indicate the presence among the studied individuals, including among patients with osteoporosis, two polymorphisms previously registered in the Genome Browser databases (www.genome.ucsc.edu/cgi-in/hgBlat) and NCBI (www.ncbi.nlm.nih.gov/snp/). New polymorphisms among the studied 57 samples were not identified. The results indicate that the T allele of the rs2228570 polymorphism showed a direct connection with osteoporosis, and this factor is associated with the risk of developing the disease and the presence of the C allele is protective.

Key words: Exon, vitamin D receptor (VDR) gene, osteoporosis, polymorphisms, sequencing.

Abbreviations: OP: osteoporosis; VDR: vitamin D receptor.

INTRODUCTION

Osteoporosis (OP) is a complex, multifactorial disorder whose pathogenesis is due to the addictive effects of various genetic determinants interacting with environmental influences and lifestyle habits (Marini et al., 2018; Hirano, 2018).

One of the most promising approaches in molecular genetics in the study of OP is the search for candidate genes associated with the disease. Particular importance is the search for candidate genes responsible for the synthesis of proteins affecting bone metabolism. Among these genes, one can distinguish the vitamin D receptor gene, which has a significant effect on bone metabolism (Hirano, 2018; Peacock et al., 2002; Mishra and Santosh, 2018).

The regulation of phosphorus-calcium metabolism is carried out with the direct participation of the active form of vitamin D (calcitriol 1.25 (OH) 2 D3) with cellular receptors. Calcitriol receptor, attributed to the group of nuclear transcription proteins, takes part not only in the process of transcription, but also in the mechanism of post-transcription, under the control of microRNA. The site responsible for coding the vitamin D receptor is located on chromosome 12 (region 12q13) (Mishra and Santosh, 2018; Seye et al., 2013; Plekhova et al., 2018).

The aim of this research is to conduct a directional search for new SNP variants in the second exon of the vitamin D receptor (VDR) gene in osteoporosis.

MATERIALS AND METHODS

The study group included 57 people - representatives of the Uzbek population, which included 42 patients with OP at the age of 40 to 65 years old (average age 53.3 ± 6.4 years), which amounted to 73.7% and 15 healthy individuals at the
age of 36 to 75 years old (average age 61.0 ± 7.8 years), with no history of fractures, which amounted to 26.3% of the total number of subjects. The diagnosis of OP was established according to the standard on the basis of data from clinical and densitometric studies.

The diagnosis of OP was established on the basis of x-ray and densitometric studies, Research Institute of Traumatology and Orthopedics of the Ministry of Health of the Republic of Uzbekistan.

DNA isolation was performed using the AmpIPrime Riboprep DNA Extraction Kit (Next Bio Bio, Russia) in accordance with the manufacturer's instruction. Determination of the concentration of the obtained preparation of nucleic acids in the samples was performed spectrophotometrically using a NanoDrop-2000 instrument (USA).

The sequenced region of the VDR gene is exon 2, consisting of 267 mon and contains the known Fokl polymorphism. The design of primers for standard PCR was carried out using the methods of bioinformatics data analysis of the NCBI database and Genome Browser using the BioEdit computer program ([2a]). Primer for driving and return chains is: 2exon_F: 3'-AGCTGGCCCTGGCAGCTGACTGTGCTCT-5' and 2exon_R: 3'-ATGGAAACACCTTGCTTCTCCTC-5'.

PCR amplification was performed on a programmable thermal cycler from Applied Biosistems-2720 (USA) in a volume of 25 μl. The composition of the reaction mixture included: 12.1 μl of ddH2O, 2.5 μl of 10×PCR buffer, 2.5 μl of a 25 mM MgCl2, 2.5 mixture of dNTP (dNTP mix, 10 mm of each) 1 μl (10 mm) of primers 2exon_F and 2exon_R, 0.4 μl (2 units) of Taq polymerase and 3 μl of DNA. The amplification program consisted of pre-denaturation of DNA - 5 min at 94°C, 35 amplification cycles: denaturation at 94°C - 30 s, annealing of primers at 58°C for 30 s, DNA synthesis at 72°C for 1 min and the final stage of the synthesis at 72°C - 10 min. The presence of PCR products was checked by electrophoresis in a 2.5% agarose gel, followed by staining with ethidium bromide and visualization in transmitted ultraviolet light using the UVT-1 transilluminator (Biocom, Russia) (Figure 1).

**Figure 1:** Detection of 2 exons of the VDR gene on electrophoregram.

The resulting cyclic sequencing products were purified from uncleaved nucleotides and primers using the Biospin PCR Purification kit (China) in accordance with the manufacturer's protocol. The purity and amount of purified PCR products was determined on a NanoDrop 2000 instrument (USA).

The first step of the cyclic sequencing reaction was to amplify a fragment (12q13.11; 47879122-47878872) of the VDR gene locus using the BigDye® Terminator v3.1 Cycle Sequencing Kit and primers for driving chain 2 exon_F, 3'-AGCTGGCCCTGGCAGCTGACTGTGCTCT-5', for the 2exon_R reverse circuit: 5'-ATGGAAACACCTTGCTTCTCCTC-3' introduced into the reaction medium separately. The reaction mixture consisted of: 5 μl of ddH2O, 1 μl of 5 x sequencing buffer, 2 μl of the final reaction mixture (dNTP, ddNTPs (Big Dye terminators) and polymerase), 1 μl of primer, and 1 μl of purified PCR product. The temperature-time mode of the cyclic sequencing reaction was from pre-denaturation of DNA - 1 min at 96°C, 25 amplification cycles: denaturation at 96°C - 10 s, primer annealing at 50°C for 30 s, DNA synthesis at 60°C for 4 min and the final stage of the synthesis at 72°C - 3 min.

The PCR product was purified from uncleaved nucleotides and primers using the BigDye XTerminator® Purification Kit according to the manufacturer’s instruction. Thereafter, the purified products of cyclic sequencing were subjected to denaturation for 2 min at a temperature of 96°C, followed by cooling. Electrophoretic separation and laser detection of cyclic sequencing products was performed on an ABI 3500 DNA Analyzer 8-capillary automatic sequencer.
**RESULTS AND DISCUSSION**

Sequencing of the fragment (12q13.11; 47879122-47878872) of the VDR gene of 57 DNA samples (42 patients with OP and 15 healthy individuals) indicate the presence of 2 polymorphisms (Figure 2). Comparison with the Genome Browser polymorphism databases (www.genome.ucsc.edu/cgi-in/hgBlat) and NCBI (www.ncbi.nlm.nih.gov/snp/) defined them as rs2228570 and rs2228572 polymorphisms.

The results suggest that two phenotypically significant polymorphisms have been identified. Comparison with the bioinformatic polymorphism base genome.ucsc.edu/cgi-in/hgBlat, ncbi.nlm.nih.gov/snp/ established their belonging to the rs2228570 and rs2228572 polymorphisms earlier described (Figure 2). Table 1 and Figure 3 shows the results obtained in the course of the study.

The frequency of the unfavorable T allele of the rs2228570 polymorphism was higher in patients with OP as compared with healthy volunteers - 20.2 and 5.9%, respectively. The frequencies of CC, CT and TT genotypes in the group of patients with OP were 64.3, 30.9, and 4.8%, respectively. The genotypic indices CC, CT and TT in the control group were 88.2, 11.8 and 0%, respectively. During the analysis of the C allele of the rs2228572 polymorphism, insignificant differences were found in the frequency of patients with OP compared with the relatively healthy individuals - 94.0 and 96.7%, respectively, in contrast to the T allele, for which the values of these indicators were - 6.0 and 3.3%. The frequencies of CC, CT and TT genotypes in the group of patients with OP were 88.1, 11.9, and 4.8%, respectively. Genotypic indices CC, CT and TT in the control group were 93.3, 6.7 and 0%, respectively. Table 2 shows the associative connection between the rs2228570 and rs2228572 polymorphisms of the VDR gene and the risk of developing OP.

A difference was found between the main group of patients with OP and the control group: in the presence of the T allele of the rs2228570 polymorphism of the VDR gene, the risk of developing OP is 1.32 times higher than in the control group. Analysis of the rs2228572 polymorphism showed a slight tendency to increase the risk for the T allele, in the absence of statistically significant differences.

The search for an associative connection between the rs2228570 polymorphism of the VDR gene and the risk of developing OP showed (Table 2) that the presence of the C allele leads to a significant ($\chi^2 = 3.69; p = 0.05$) decrease in the probability of disease (RR = 0.76; 95% CI: 0.62–0.93).
The study of the genotypes of the rs2228570 polymorphism of the VDR gene showed the presence of an associative link in the TT-genotype, in which the risk of developing OP increases by 1.56 times (95% CI: 1.24–1.95), and for heterozygotes with CT, the genotype is not statistically significant 1.35 times higher (95% CI: 1.00–1.82).

At the same time, the chance of detecting an unfavorable genotype in the main group in the study of the genotype ST rs2228572 was not significantly higher as compared to the control group (RR = 1.15; 95% CI: 0.77–1.71), and for CC homozygotes, a statistically insignificant lower probability of OP risk was found (RR = 0.87; 95% CI: 0.59–1.29). Thus, it shows the presence of a direct connection with the
disease for the T allele of the rs2228570 polymorphism, while this factor is statistically significantly associated with the risk of developing OP, and the presence of the C allele is protective.

The functionally unfavorable T rs2228570 allele of the VDR gene significantly contributes to the formation of OP. The risk of developing the pathology in the presence of this allele may increase by 1.32 times, and the chances of disease formation, if present, increase by more than 4.0 times ($\chi^2 = 3.69; p < 0.05; OR = 4.06; 95\% CI 0.88-18.65$).

The literature with specific studies of the rs2228570 and rs2228572 polymorphisms of the VDR gene in patients with OP is practically absent. The number of studies on the role of polymorphisms of VDR genes in the formation of OP is small, and their results seem ambiguous (Plekhova et al., 2018).

However, despite a certain fragmentation, such studies in the future open up new horizons in identifying the pathogenesis of OP. We consider it necessary to conduct further studies of other genes of OP markers, which would allow to link together genetic and other mechanisms of OP development and to develop not only effective ways to predict the development and course of this disease, but also to identify promising methods for their personalized therapy.

The FokI polymorphism is located in the second exon of the VDR gene. Replacement of T> C in position leads to the loss of the first start-codon (ATC> ACG). mRNA is lost, and the synthesis of the product begins with the second start of the codon (AUG> ACG) (Bolamperti et al., 2013). Derived by Arai et al. (1997), the results suggest that the short variant of the protein (424 amino acid residues, "T" allele) encoded by the VDR gene is 1.7 times more active than the longer variant of the protein (Maltsev, 2014).

According to Jurutka et al. (2000), it is known that due to more efficient interaction with the transcription factor IIIB (TFIIB), a short variant of the protein encoded by the VDR gene has a higher activity (Falcon-Ramirez et al., 2016).

Thus, the obtained and processed preliminary data analyzing the results of the sequence of VDR gene polymorphisms showed a very low probability of finding new polymorphisms in the second exon at OP and, accordingly, the inefficiency of finding new polymorphisms in this part of the vitamin D gene.

Conclusions

The following conclusions are drawn from the study:

1) The functional adverse T allele and the rs2228570 TT genotype of the VDR gene significantly contribute to the formation of osteoporosis;
2) The presence of rs2228570 C-allele of the VDR gene is protective;
3) The homozygous T / T genotype is not characteristic of our population as indicated by the results of the study of the rs2228572 polymorphism in the control and main groups and rs2228570 of the VDR gene in the control group in this sample.

ACKNOWLEDGEMENTS

The authors are grateful to the scientific adviser and the head of the Department of Molecular Medicine and Cell Technologies, Scientific Research Institute of Hematology and Blood Transfusion of the Ministry of Health of the Republic of Uzbekistan, Hamid Yakubovich Karimov and the head of the laboratory of medical genetics, Kodirzhon Tukhtabaevich Boboev for assistance in the management and organization of the conducted studies.

REFERENCES


