DOI 10.31489/2023BMG1/117-122

УДК 575.224.22

V.V. Protas[1]*, G.P. Pogossyan[1], K.G. Li[1], M.P. Danilenko[2]

[1]*Karagandy University of the name of academician E.A. Buketov, Karaganda, Kazakhstan;*
[2]*Ben-Gurion University of the Negev, Beersheba, Israel*
**Corresponding author: akimasix@mail.ru*

Frequency of rs2228570 single nucleotide polymorphism of Vitamin-D Receptor (VDR) gene among the Kazakh ethnic group

The article presents the results of the study of vitamin D receptor (VDR) gene rs2228570 single nucleotide polymorphism (SNP) genotypes and individual alleles frequency among the Kazakh ethnic group representatives living in the Karaganda region. This SNP was determined by real-time polymerase chain reaction using TaqMan technology. The study relevance is due to the fact that genetic variations in rs2228570 affect the synthesis of the VDR protein and its activity as a transcription factor that regulates the expression of other genes. This mechanism determines the association of individual rs2228570 genotypes or alleles with susceptibility, course, and outcomes of various diseases. The polymorphism frequency may be depending on ethnicity. According to the study results, the most common genotypes of rs2228570 SNP among the Kazakhs were AG (32.8 %) and GG (25.2 %). The rarest are homozygotes TT (1.7 %) and CC (0.8 %). The frequency of all rs2228570 alleles was detected simultaneously in the present study for the first time. G became the predominant allele (51.3 %), less common was A (31.0 %), and the C and T alleles were the rarest (11.8 % and 5.9 %, respectively). The potential value of this SNP further study as a possible factor influencing the body's susceptibility to various diseases, including COVID-19, is shown.

Keywords: vitamin D receptor (VDR) gene, VDR gene single nucleotide polymorphism, rs2228570, genotype frequency, allele frequency, Kazakh ethnic group, vitamin D, vitamin D receptor.

Introduction

Vitamin D is well known for its role in maintaining calcium and phosphorus homeostasis. It enters the human body in two ways: through exogenous intake of food rich in vitamin D, or through its endogenous synthesis in the skin under the influence of ultraviolet B (UVB) radiation. Both forms of vitamin D are inactive, so its two-step activation follows: first in the liver (forming 25(OH)D or calcidiol) and then in the kidneys (forming 1.25-dihydroxyvitamin D; $1.25(OH)_2D_3$ or calcitriol) [1].

Calcitriol is a hormonally active form of vitamin D and has a wide range of extraskeletal biological responses, including inhibition of the progression of breast, large intestine and prostate cancer cells, protection against a number of autoimmune diseases, including multiple sclerosis and inflammatory bowel disorder [2]. Also, there are many studies that prove the participation of vitamin D in the control of blood pressure, reducing the risk of type I and II diabetes, multiple sclerosis, rheumatoid arthritis, heart disease and infectious diseases, as well as in the regulation of immune responses [3]. All biological extraskeletal effects of calcitriol are mediated by the nuclear vitamin D receptor (VDR), which belongs to the family of steroid receptors. After binding to calcitriol, VDR heterodimerizes with the retinoid X receptor (RXR) and moves to the nucleus, where it binds to the VDR sensitive element (VDRE) in target genes and controls the expression of more than 500 genes [4]. At the same time, the activity and functioning of VDR depends on the structure of the VDR gene located on chromosome 12q13.1 [5].

The VDR gene is polymorphic, with over 470 single nucleotide polymorphisms (SNPs) identified. One of the most common and studied SNPs of the VDR gene is rs2228570 [6]. A number of studies have revealed the association of individual genetic variations in rs2228570 with diseases such as dengue, bronchial asthma, hepatitis B, Parkinson's disease and tuberculosis [7–12]. There are also suggestions about its role in susceptibility to COVID-19 [13, 14]. These facts make rs2228570 an important potential object for study.

Thus, in this study, for the first time, the frequencies of genotypes and all four alleles of the VDR gene rs2228570 among representatives of the Kazakh ethnic group were studied.

Experimental

In total, 119 samples of biological material (blood) taken from adult representatives of the Kazakh ethnic group living in the city of Karaganda and the Karaganda region were examined. The age range was 18–77 years old (mean age is 43). The gender distribution was 39 men and 80 women.

Blood was withdrawn into an EDTA tube. The extraction of DNA from the blood samples was performed by "RIBO-prep" (Amplisens, Russia) according to the kit manufacturer's protocol. Genotyping of VDR gene SNP rs2228570 was carried out by real-time polymerase chain reaction (Real-Time PCR) using TaqMan probes (Lumiprobe, Russia). The sequences of primers and probes, as well as the PCR conditions, are shown in Table 1. All samples were analyzed on a Real-Time PCR DTlite instrument (DNA-Technology, Russia).

Table 1

The corresponding data for rs2228570 genotyping

		Sequence (5'–3')	PCR protocol (denaturation, cycles, extension)
Primers			94 °C / 3 min (94 °C / 15 sec, 62 °C / 30 sec) × 40
	forward	5'- TCCACACACCCCACAGATCC-3	
	reverse	5'- GTGGGTGGCACCAAGGATG-3'	
TaqMan			
	probe A	5'-CCGCCATTGCCTCCATCCCTGTAAGAA-3'	
	probe C	5'-CCGCCATTGCCTCCCTCCCTGTAAGAA-3'	
	probe G	5'-CCGCCATTGCCTCCGTCCCTGTAAGAA-3'	
	probe T	5'-CCGCCATTGCCTCCTTCCCTGTAAGAA-3'	

The categorical variables are described as percentages. The chi-square test (χ^2 test) was used to compare categorical data. $P < 0.05$ was considered statistically significant. All genotypes were tested for Hardy–Weinberg equilibrium using chi-squared test. The statistical analyses were carried out by the GraphPad Prism 8.0 program (Graph-Pad Software, CA, USA).

Results and Discussion

Previously, in review article, we compared rs2228570 frequency alleles and genotypes submitted in dbSNP NCBI (National Center for Biotechnology Information, USA) with data published in peer-reviewed publications [15]. It was found that rs2228570 is four-allelic, with a predominance of G (0.611257) and A (0.388743) alleles [16]. However, most researchers simultaneously analyzed the occurrence of only two of the four alleles in the following combinations: C>T or A>G [7–12]. In the present study, we simultaneously determined all possible variants of the rs2228570 genotypes. The observed rs2228570 genotype frequencies were consistent with Hardy–Weinberg equilibrium with p values greater than 0.05 ($p = 0.9638$).

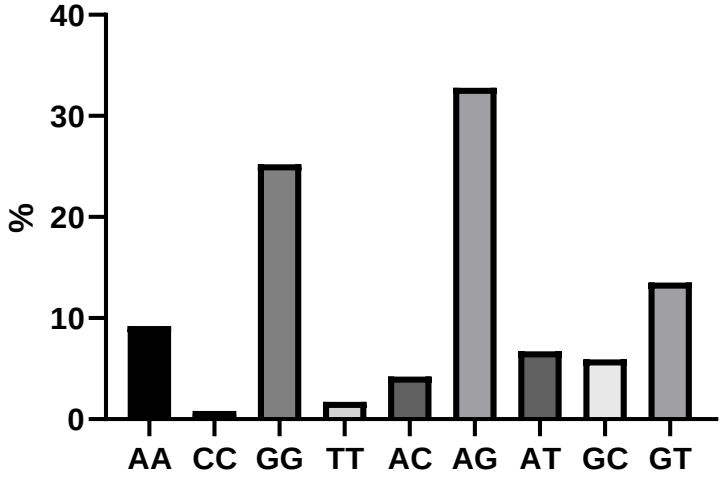

Figure 1. Frequency of rs2228570 genotypes in the Kazakh population

As can be seen from Figure 1, the most common genotypes among representatives of the Kazakh ethnic group are AG (32.8 %) and GG (25.2 %). Frequencies vary from 13.5 % to 4.2 % in GT, AA, AT and GC genotypes. The rarest were TT and CC homozygotes (1.7 % and 0.8 %, respectively). The obtained data coincide with dbSNP in that genotypes with G and A alleles predominate [16].

Despite the large number of publications that studied the occurrence of CC, CT and TT genotypes [7–11], it was not possible to compare them with the data obtained, since the frequency of these genotypes in the Kazakh population is critically low. At the same time, data on the frequency of AC, AT, GC and GT genotypes is practically absent in the literature.

Therefore, only the frequencies of the AA, AG, and GG genotypes (in % of the total number of occurrences; $n = 80$) could be compared with the results of other authors (Table 2).

Table 2

Comparative analysis of AA/AG/GG genotypes frequency of the VDR gene rs2228570

Ethnicity / country	Genotype (%)			P-value	Reference
	AA	AG	GG		
KZ	*14*	*49*	*37*		
UAE	27	42	31	NS	Osman et al. [6]
UK	48	41	11	****	Bid et al. [17]
French	43	47	10	****	Zmuda et al. [18]
Japan	37	51	12	****	Bhanushali et al. [19]
North Indian	44	49	7	****	Tokita et al. [20]
India	59	36	5	****	Bid et al. [17]
**** = $p < 0.0001$; NS = not significant.					

Table 2 presents the comparative data on three rs2228570 genotypes between the Kazakh population and others. It can be seen that a statistically significant difference was not found only in comparison with the UAE population [6], in all other cases it was maximum ($p < 0.0001$).

The results of the analysis of individual rs2228570 alleles frequency is shown in Figure 2.

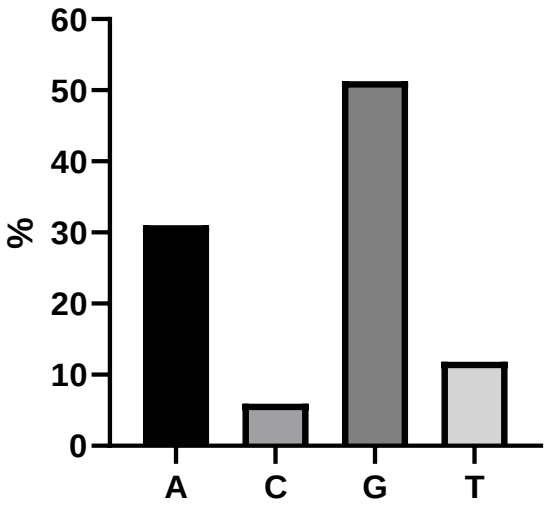

Figure 2. Frequency of rs2228570 alleles in the Kazakh population

It was found that the most common allele among the Kazakh population is G (51.3 %), and the A allele is 20 % less common (31.0 %). The data obtained for two alleles are fully consistent with dbSNP (assembly 155 dated April 9, 2021, aggregated data with sampling of 236272 samples) [16]. The occurrence of T and C alleles was 11.8 % and 5.9 %, respectively, which is significantly higher than the dbSNP data (T=0.000000, C=0.000000) [16], but inferior to the results of other studies [7, 11]. The comparative analysis of the occurrence of A and G major alleles (in % of the total number of occurrences; $n = 196$) is presented in Table 3.

Table 3

Comparative analysis of A and G alleles frequency of VDR gene rs2228570

Ethnicity / country	Alleles (%)		P-value	Reference
	A	G		
KZ	*37.8*	*62.2*		
dbSNP total	38.9	61.1	NS	[16]
dbSNP European	38.7	61.3	NS	[16]
dbSNP Asian	43.8	56.2	NS	[16]
UAE	48.04	51.96	NS	Osman et al. [6]
UK	68.50	31.50	****	Bid et al. [17]
French	66.50	33.50	****	Zmuda et al. [18]
Japan	62.50	37.50	****	Bhanushali et al. [19]
North Indian	68.50	31.50	****	Tokita et al. [20]
India	77.00	23.00	****	Bid et al. [17]
**** = $p < 0.0001$; NS = not significant				

As it can be seen from the Table 3, the data on the prevalence of individual rs2228570 alleles obtained in this study do not have statistically significant differences with the generalized dbSNP NCBI data, as well as separate results for European and Asian ethnic groups [16]. Also, the results are commensurate with those for the population of the United Arab Emirates [6]. At the same time, there is a fundamental discrepancy with the results of individual studies [17–20]. This can be explained by errors in these studies due to the fact that they did not take into account two more possible alleles of rs2228570 (C and T).

Thus, this paper presents unique data on the overall prevalence of VDR gene rs2228570 genotypes and four alleles among representatives of the Kazakh ethnic group.

Conclusions

After examination of the DNA samples of 119 Kazakhs living in the Karaganda region, it was found that the most common genotypes of the rs2228570 single nucleotide polymorphism of the VDR gene are AG and GG. The rarest genotypes are TT and CC. Accordingly, G became the predominant allele, A allele was slightly less common, and the C and T alleles were the rarest. The results obtained are of interest as a general population study and as a material for comparison. In addition, in the future, it is possible to predict the likelihood and course of diseases associated with certain genetic variations of VDR gene rs2228570 SNP among Kazakhs. It is also planned to test the potential impact of this SNP for susceptibility to COVID-19.

References

1 Bouillon, R., Marcocci, C., Carmeliet, G., Bikle, D., J. & White H. (2019). Skeletal and extraskeletal actions of vitamin D: current evidence and outstanding questions. *Endocr. Rev., 40,* 1109–1151. https://doi.org/10.1210/er.2018–00126

2 Christakos, S., Dhawan, P., Verstuyf, A., Verlinden, L. & Carmeliet, G. (2016). Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. *Physiol Rev., 96(1),* 365–408. https://doi.org/10.1152/physrev.00014.2015

3 Hewison, M. (2010). Vitamin D and the intracrinology of innate immunity. *Mol Cell Endocrinol, 321,* 103–111. https://doi.org/10.1016/j.mce.2010.02.013

4 Bradley, R., Schloss, J., Brown, D., Celis, D., Finnell, J. & Hedo, R., (2020). The effects of vitamin D on acute viral respiratory infections: A rapid review. *Adv Integr Med., 7(4),* 192–202. https://doi.org/10.1016/j.aimed.2020.07.011

5 Taymans, S.E., Pack, S., Pak, E., Orban, Z., Barsony, J., Zhuang, Z. & Stratakis, C.A. (1999). The human vitamin D receptor gene (VDR) is localized to region 12cen-q12 by fluorescent in situ hybridization and radiation hybrid mapping: Genetic and physical VDR map. *J Bone Miner Res, 14 (7),* 1163–1166. https://doi.org/10.1359/jbmr.1999.14.7.1163

6 Osman, E., Al Anouti, F., El Ghazali, G., Haq, A., Mirgani, R. & Al Safar, H. (2015). Frequency of rs731236 (Taql), rs2228570 (Fok1) of Vitamin-D Receptor (VDR) gene in Emirati healthy population. *Meta Gene, 6, 49–52.* DOI: https://doi.org/10.1016/j.mgene.2015.09.001

7 Alagarasu, K., Honap, T., Mulay, A.P., Bachal, R.V., Shah, P.S. & Cecilia, D. (2012). Association of vitamin D receptor gene polymorphisms with clinical outcomes of dengue virus infection. *Human Immunology, 73(11),* 1194–1199.

8 Hu, W., Wang, L., Chen, B., & Wang, X. (2020). Vitamin D receptor rs2228570 polymorphism and Parkinson's disease risk in a Chinese population. *Neuroscience Letters, 717,* 134–152. https://doi.org/10.1016/j.neulet.2019.134722

9 Kresfelder, T.L., Janssen, R., Bont, L., Pretorius, M. & Venter, M. (2011). Confirmation of an association between single nucleotide polymorphisms in the VDR gene with respiratory syncytial virus related disease in South African children. *J Med Virol, 83 (10),* 1834–1840. https://doi.org/10.1002/jmv.22179

10 Grzegorzewska, A.E., Ostromecki, G., Zielińska, P., Mostowska, A. & Jagodziński, P.P. (2014). T-cell cytokine gene polymorphisms and vitamin D pathway gene polymorphisms in end-stage renal disease due to type 2 diabetes mellitus nephropathy: comparisons with health status and other main causes of end-stage renal disease. *J Diabetes Res, 2014,* 1203–1217. https://doi.org/10.1155/2014/120317

11 Bahrami, A., Parsania, M., Pourfatholl, A.A., Haghighat, A. & Sharifi, Z. (2020). Association of VDR (rs2228570, rs731236, rs7975232, rs1544410) and DBP (rs7041) genes polymorphisms with chronicity of hepatitis B in Iranian patients. *Gene Reports, 19,* 1006–1015.

12 Lee, S.W., Chuang, T.Y., Huang, H.H., Liu, C.W., Kao, Y.H. & Wu, L.S. (2016). VDR and VDBP genes polymorphisms associated with susceptibility to tuberculosis in a Han Taiwanese population. *J Microbiol Immunol Infect, 49 (5),* 783–787. https://doi.org/10.1016/j.jmii.2015.12.008

13 Jafarpoor, A., Jazayeri, S.M., Bokharaei-Salim, F., Ataei-Pirkooh, A., Ghaziasadi, A. & Soltani. S. (2022). VDR gene polymorphisms are associated with the increased susceptibility to COVID-19 among iranian population: A case-control study. *Int J Immunogenet., 49(4),* 243–253. https://doi.org/10.1111/iji.12585

14 Apaydin, T., Polat, H., Dincer Yazan, C., Ilgin, C. & Elbasan, O. (2022) Effects of vitamin D receptor gene polymorphisms on the prognosis of COVID-19. *Clin Endocrinol (Oxf), 96(6),* 819–830. https://doi.org/10.1111/cen.14664

15 Protas, V.V., Pogossyan, G.P., Li, K.G., & DanilenkoM.P. (2021) Vitamin D receptor gene polymorphisms characteristic. *Bulletin of the Karaganda University. Series "Biology. The medicine. Geography", 4(104),* 60–70. https://doi.org/10.31489/2021BMG4/60–70

16 Retrieved from https://www.ncbi.nlm.nih.gov/snp/rs2228570? horizontal_tab=true#frequency_tab

17 Bid, H.K., Mishra, D.K. & Mittal R.D. (2005). Vitamin-D receptor (VDR) gene (Fok-I, Taq-I and Apa-I) polymorphisms in healthy individuals from north Indian population. *Asian Pac J Cancer Prev, 6 (2),* 147–152.

18 Zmuda, J.M., Cauley, J.A., Danielson, M.E., Wolf, R.L. & Ferrell R.E. (1997) Vitamin D receptor gene polymorphisms, bone turnover, and rates of bone loss in older African-American women. *J. Bone Miner. Res., 12 (9),* 1446–1452. https://doi.org/10.1359/jbmr.1997.12.9.1446

19 Bhanushali, A.A., Lajpal, N., Kulkarni, S.S., Chavan, S.S. & Bagadi, B.R. (2009) Das Frequency of fokI and taqI polymorphism of vitamin D receptor gene in Indian population and its association with 25-hydroxyvitamin D levels. *Indian J. Hum. Genet., 15 (3),* 108–113. https://doi.org/10.4103/0971–6866.60186

20 Tokita, A., Matsumoto, H., Morrison, N.A., Tawa, T., Miura, Y. & Fukamauchi, K. (1996) Vitamin D receptor alleles, bone mineral density and turnover in premenopausal Japanese women. *J. Bone Miner. Res., 11 (7),* 1003–1009. https://doi.org/10.1002/jbmr.5650110718

В.В. Протас, Г.П. Погосян, К.Г. Ли, М.П. Даниленко

Қазақ этникалық тобының өкілдері арасында бірнуклеотидті полиморфизмнің rs2228570 D дәрумені рецепторы генінің (VDR) кездесу жиілігі

Мақалада Қарағанды облысында тұратын қазақ этникалық тобының өкілдері арасында бірнуклеотидті полиморфизмнің (SNP) rs2228570 генотиптерімен жекелеген аллельдерінің D дәрумені рецепторы генінің (VDR) жиілігін зерттеу нәтижелері келтірілген. Берілген SNP-ді анықтау TaqMan технологиясын қолдана отырып, нақты уақыт режимінде полимеразды тізбекті реакция әдісімен жүзеге асырылды. Зерттеудің өзектілігі rs2228570 генетикалық вариациялары D дәрумені рецепторы ақуызының синтезіне және оның басқа гендердің экспрессиясын реттейтін транскрипциялық фактор ретіндегі белсенділігіне әсер ететіндігіне байланысты. Бұл механизм rs2228570 жеке генотиптерінің немесе аллельдерінің әртүрлі аурулардың сезімталдығымен, ағынымен және нәтижелерімен байланысын анықтайды. Осы полиморфизмнің кездесу жиілігі этникалық ерекшеліктеріне байланысты айтарлықтай өзгеруі мүмкін. Зерттеу нәтижелері бойынша қазақтар арасында VDR генінің rs2228570 бірнуклеотидті полиморфизмінің ең жиі кездесетін генотиптері AG (32,8 %) және GG (25,2 %) болды. Ең сирек кездесетіні — TT (1,7 %) және CC (0,8 %) гомозиготалары. Осы зерттеуде rs2228570 барлық төрт аллельдің таралуы алғаш рет бір уақытта анықталды. Басым аллель G (51,3 %) болды, A (31,0 %) — сирек кездеседі, ал C және T аллельдері ең сирек кездеседі (сәйкесінше 11,8 % және 5,9 %). Rs2228570 ағзаның әртүрлі ауруларға, соның ішінде COVID-19-ға бейімділігіне әсер ететін ықтимал фактор ретінде одан әрі зерттеудің ықтимал маңыздылығы көрсетілген.

Кілт сөздер: D дәрумені рецепторының (VDR) гені, VDR генінің бірнуклеотидті полиморфизмі, rs2228570, генотиптердің кездесу жиілігі, аллельдердің кездесу жиілігі, қазақ этникалық тобы, D дәрумені, D дәрумені рецепторы.

В.В. Протас, Г.П. Погосян, К.Г. Ли, М.П. Даниленко

Частота встречаемости однонуклеотидного полиморфизма rs2228570 гена рецептора витамина D (VDR) среди представителей казахской этнической группы

В статье представлены результаты исследования частоты встречаемости генотипов и аллелей однонуклеотидного полиморфизма (SNP) rs2228570 гена рецептора витамина D (VDR) среди представителей казахской этнической группы, проживающих в Карагандинской области. Генотипирование данного SNP осуществлялось методом полимеразной цепной реакции в режиме реального времени с использованием технологии TaqMan. Актуальность исследования обусловлена тем, что генетические вариации rs2228570 влияют на синтез белка-рецептора витамина D и его активность как транскрипционного фактора, регулирующего экспрессию других генов. Данный механизм обусловливает ассоциацию отдельных генотипов или аллелей rs2228570 с восприимчивостью, течением и исходами различных заболеваний. Частота встречаемости данного полиморфизма может сильно варьироваться в зависимости от этнической принадлежности. По результатам исследования, наиболее часто встречаемыми генотипами однонуклеотидного полиморфизма rs2228570 гена VDR среди казахов стали AG (32,8 %) и GG (25,2 %). Самыми редкими — гомозиготы TT (1,7 %) и CC (0,8 %). Следует отметить, что в настоящем исследовании впервые была одновременно выявлена распространенность всех аллелей rs2228570. Преобладающей аллелью стала G (51,3 %), менее распространенной — A (31,0 %), а аллели C и T — наиболее редко встречаемые (11,8 % и 5,9 % соответственно). Показано потенциальное значение дальнейшего изучения rs2228570 как возможного фактора, влияющего на восприимчивость организма к различным заболеваниям, в том числе и COVID–19.

Ключевые слова: ген рецептора витамина D (VDR), однонуклеотидный полиморфизм гена VDR, rs2228570, частота встречаемости генотипов, частота встречаемости аллелей, казахская этническая группа, витамин D, рецептор витамина D.