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POLYMORPHIC VARIANTS OF GENES TYPE I COLLAGEN (*COL1A1*), CALCITONIN RECEPTORS (*CALCR*) AND VITAMIN D (*VDR*) AND THE PARAMETERS OF TEENAGERS MUSCULOSKELETAL SYSTEM IN THE PRIMORSKY TERRITORY

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The physiological parameters of the musculoskeletal system status and the prevalence of nucleotide sequence variants genes collagen *COL1A1* rs1800012 IVS1 c.2046 G> T, and receptors of calcitonin *CALCR* rs1801197 c.1377 T> C and the intracellular vitamin D *VDR* rs731236 c.1056 T> C in healthy teenagers of the Primorsky Territory was studied. It was shown that the homozygous TT genotype of the gene TaqI VDR is 5 times more prevalent among girls than among boys (19.6% and 3.9%, respectively). Moreover, in carriers of homozygous TT and heterozygous CT genotypes of this gene, fractures were more common (p = 0.051, p = 0.012) than in carriers of SS genotype. It was established that, in carriers of the heterozygous genotype GT of the gene *COL1A1* fractures and impaired posture were significantly more. No correlation was found between the presence of fractures and carriers of the genotype TT of the *CALCR* calcitonin receptor (p = 0.143). In the examined cohort of the teenagers are predominant the carriers of the heterozygous genotypes Ss (GT) (87.3%, the collagen gene *COL1A1*) and CT (80% - the calcitonin receptor gene and 66, 7% - the intracellular vitamin D receptor gene). In carriers of the heterozygous Ss (GT) *COL1A1* gene, the presence of the heterozygous CT genotype of calcitonin receptor genes (*CALCR*) and vitamin D *VDR* was more common. Thus, genetic testing made it possible, firstly, to identify the prevalence of “unfavorable” alleles and their combinations in the studied genes (from 3.8% to 50%) that regulate calcium metabolism in teenagers; secondly, to determine the relationship between genotypes and the presence of fractures and impaired posture, which makes it possible to distinguish a group of subjects with a high risk of developing MSS diseases.

Keywords: gene of type 1 collagen, gene of calcitonin receptor, gene of vitamin D receptor, musculoskeletal system.

Introduction. In adolescence, along with the restructuring of the hormonal system, the final formation of the musculoskeletal system occurs, which is accompanied by a maximum increase in the mineral density of bone tissue [1, 6]. It has been shown that metabolic contravention in bone formation lead to inhibition of the linear growth of children and can cause scoliosis and juvenile osteoporosis [3, 9]. In the structure of functional and chronic diseases in children, the frequency of disorders and diseases of the musculoskeletal system (MSS) is in the I – III place, and according to some data, 67% determine the second group of health associated with its disorganization [5, 10]. The activity of bone remodeling that occurs during prepubertal period depends on the degree of expression of genes initiating the synthesis of various proteins [2]. Such proteins include matrix proteins (type I collagen), regulatory proteins that take part in calcium metabolism, cytokines, growth factors and their receptors, as well as bone metabolism enzymes.

One of the main regulators of calcium-phosphorus metabolism in the body is calcitonin, which is synthesized in parafollicular cells of the thyroid gland and has an inhibitory receptor-mediated effect on the activity of osteoclasts, reducing the rate of bone resorption [8]. The calcitonin receptor gene CALCR is located on chromosome 7q21.3 and encodes isoform 1 of the G protein subfamily (G protein-coupled receptors) [18]. Replacing cytosine with thymine (C> T) in exon 17 of the CALCR gene at position 1340 (rs1801197) leads to the replacement of proline amino acid (CCG) with leucine (CTG) at position 463 of the receptor protein molecule and, as shown, is in positive correlation with bone density [11]. Vitamin D also takes part in the process of bone tissue calcium and phosphate metabolism, the hormone-active form of which is calcitriol 1.25 (OH) 2 D3 interacting with receptors of target cells [25]. The calcitriol receptor (VDR) or NR1I1 belongs to the family of intracytosolic, nuclear, takes part in transcription and mechanisms of protein synthesis, and is encoded by a gene located on the 12th chromosome (12q13) [16, 25]. The degree of expression of this receptor determines the effect of calcitriol on bone mineral density and skeleton formation [22]. Moreover, the kinetics and degree of calcium accumulation in the puberty period depends on the presence of various variants of this VDR gene [19]. The most promising diagnostic way in determining the mechanisms of osteoporosis development is the identification of VDR gene variants in exon 2 of FokI (rs10735810), between 8 and 9 exons of BsmI (rs1544410) and TaqαI (rs731236) [23].

The interest are also data on the combination of the genes number expression involved in the post-translational modification of the bone tissue structure-forming protein — type I collagen, consisting of two polypeptide chains α1 and α2, [13, 17]. Two genes carry information about its structure: COL1A1 (gene of the α1 chain) and COL1A2 (gene of the α2 chain). It was shown that polymorphism

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variants gene COL1A1 can cause a wide range of diseases, from osteoporosis to lethal forms of imperfect osteogenesis in Ehlers-Danlo syndrome [14, 15, 17]. Thus, the role of variations in the nucleotide sequences of genes type 1 collagen, receptors calcitonin and vitamin D in the development of various osteopathologies in the adult population has been proved, and it has been demonstrated that these diseases are age-dependent [7, 20, 23, 24]. While, the ratio of the variants these genes with the parameters of the adolescents musculoskeletal system physical examination makes it possible to obtain additional information about the development of possible pathological changes. Given the known data on the predominant presence in children aged 7-17 years of the second health group associated with disorders and diseases of the MSS, there is a need to study the distribution of these genes variants. On the other hand, since a significant ethnic difference in the distribution of genetic determinants associated with the acquisition of peak bone mass and the architecture of the MSS and / or osteoporosis has been demonstrated, such a comparative study is also of certain interest [8, 14, 19, 20, 21, 23]. The purpose of this study: to determinate the prevalence of genes polymorphic variants type I collagen (COL1A1) and calcitonin receptors (CALCR) and vitamin D (VDR) in relation to the parameters of physical examination of MSS in adolescents of the Primorsky Territory.

Materials and research methods. The selection for the study was formed from the number of students undergoing planned medical inspection in the Primorsky Territory general education schools (April-May 2018). After the informed consent of the use survey data for scientific purposes was signed by parents, guardians or trustees, a clinical examination was carried out with the definition of a health group (recommendations of the Institute for Hygiene of Children and Adolescents, orders of the Ministry of Health of the Russian Federation No. 621 dated 12/30/2003 and No. 514n dated 08/10/2017). The child's development stories were also studied (form 112 / u). Assessment of physical development was carried out using regional percentile type tables. The measurement of height and weight, the shape of the chest examining the front, back and profile, posture (the difference between the measurements of the cervical and lumbar sagittal bends of the spine), the shape of the legs, physiometric indicators (vital capacity of the lungs, compressive strength of the hands, number pull-ups on the high bar for boys on the low bar for girls) was determined. Additionally, photoplantography was performed to study physical development.

Molecular genetic research. For genetic analysis, which was carried out on the basis of the Central Research Laboratory of Pacific State Medical University, DNA samples isolated from whole venous blood (volume 4 ml) by phenol-chloroform extraction using the kit DNA-sorb (AmpliSens biotechnologies, Russia). PCR amplification of DNA samples with a purity of A260/280 = 1.8 ± 0.1 and a concentration in the range of 50-100 ng/μl was performed in a volume of 25 μl. The purity index of the DNA preparation was calculated by the ratio of the optical density values at the absorption wavelengths of the samples 260/230 nm, the coefficient value from 1.8 to 2.2 was taken into account.

The identification of gene variants the collagen chain α1 COL1A1: pp.104–441G> T (rs1800012), calcitonin receptor CALCR: c.1377C> T CALCR (rs1801197) and intracellular vitamin D receptor VDR: c.1056T> C (rs731236) was carried out by polymerase chain reaction (PCR) in real time on a PicoReal amplifier (Finland). The reagent kits COL1A1-test, CALCR-test and VDR-test (GenoTehnologiya, Russia) were used. At the end of PCR amplification, according to the protocols in the instructions for the kits, the threshold cycle value (Ct) was determined from the fluorescence curve of the sample. Used oligonucleotide probes labeled with 6-carboxy-4 ', 5'-dichloro-2', 7'-dimethoxyfluorescein (6-JOE). The value of the standard deviation Ct for repetitions of each analyzed sample did not exceed 0.5.

Statistical methods. Quantitative indicators of the bodily characteristics of MSS were evaluated using Student's t-test, regression analysis of the data, and the Fisher test was calculated. The association between the variants of the studied genes and the parameters of the bodily examination was calculated by calculating the odds ratio (OR) and the corresponding 95% confidence interval (CI). Data uniformity was determined using the χ-squared Q-test, the significance of the combined OR was determined using the Z-test at $p < 0.05$. The Fisher test was used to assess agreement with Hardy-Weinberg equilibrium at a significance level of $p < 0.05$. Statistical analysis was performed using Review Manager 5.1 software.

Results and discussion. A comprehensive assessment of growth factors (106 teenagers aged 15-17 years) revealed significant gender differences in terms of length and body weight, chest circumference (OCH, $p \geq 0.005$). The distribution by the health groups of the examined individuals showed that only 2 people (1.8%) corresponded to the first group, while all the others made up the second (Table 1). Correct posture was observed in 69 children (63%), and in boys this indicator prevailed and amounted to 79 people (73%) compared to 59 (54%) in girls. Kyphotic, hyperlordosis and hyperkyphotic posture were more often observed in girls than in boys, 16 and 10 (15% and 9%) people, respectively. A straightened back (low severity of bending of the spine, with reduced mobility of the ribs, the presence of lateral curvature of the spine) was also noted more often in girls (24 people, 22%) than in boys (15, 14%). The results of photoplantography showed the presence of transverse flatfoot of the 1st degree in 29 people (27%), 2nd degree in 39 (63%), and 3rd degree in 5 (5%) of the subjects (Table 1). Longitudinal flatfoot of the 1st degree was detected in 42 people (39%), of the 2nd degree in 9 subjects (8%) and of the 3rd degree was not detected. The presence of normal foot development was noted only in 10 (9%) of the subjects. Thus, our data demonstrate the need to study the physiological parameters of the musculoskeletal system in adolescents in order to obtain timely information. Photoplantography data are of particular importance, since flat feet are known to have a negative effect on the rational distribution of load on overlying joints and lead to more serious postural disturbances [3, 5]. It is also shown that when the anthropometric parameters and indicators of the circulatory system are conjoined, children suffering from flat feet and scoliosis show an increase in heart rate and an increase in stroke and minute blood volume [6, 9].

Polymorphism of the gene COL1A1: pp. 104–441G> T in replacing guanine with thymine is functionally manifested by the disturbance of the transcription factor binding in the region of the first intron. The inheritance of the COL1A1 gene mutation is autosomal dominant and is found in men and women with the same frequency. The probability of the occurrence of MSS disease (osteoporosis, a history of bone fractures) in children, subject to the inheritance of a mutation of this gene from one of the parents, is 50%. According to the data of the genetic study, we divided the subjects into groups according to the SS (GG, genotype 0), ss (TT, genotype 2), and Ss (GT, genotype 1) genotypes. The number of carriers

of the heterozygous genotype (Ss) was 37.3% in boys and 53.0% in girls; the homozygous SS (GG) genotype in 2.0% and 7.8%, respectively, and the ss (TT) genotype was not detected (Table 2). It was shown that the carriers distribution of genotypes depending on the presence of the knee joint ligaments break (GG-82.2% GT-16.7%, TT-1.1%) significantly differed (p = 0.036) from the group of subjects without this pathology (GG -71.4% GT-26.5%, TT-2.2%) [22]. In our study, carriers of the heterozygous Ss (GT) of the gene COL1A1 were significantly more likely to have fractures (p = 0.12, Table 3) than carriers of the homozygous Ss (GT) genotype. The distribution of carriers genotypes COL1A1:pp. 104–441G> T, respectively of the incorrect posture also showed a significant difference between the groups of subjects (p = 0.07 and p = 0.0003, table 4).

The single nucleotide replacement of thymine with cytosine (T> C) in 17 exon of the calcitonin receptor gene (CALCR) at position 1340 (rs1801197) can lead to a change in the functional activity of the encoded protein. This change in osteoclastic receptors is manifested by activation of the bone resorption process and the development of osteoporosis, the presence of which is more often observed among carriers of the variant polymorphism of the homozygous form of SS [16]. The predominance of heterozygous TS (80.4%) in the study of the gene calcitonin receptor polymorphic variants rs 1801197 CALCR: c.1377C> T p. P447L was determined, while homozygous CC and TT genotypes were found in 13.7% and 5.9% (Table 2). Despite the known data on the predominant disease with osteoporosis by carriers of a homozygous form of SS, we did not establish a relationship between the presence of fractures and carriers of this genotype, while a significant relationship (p = 0.14, Table 3) was found for carriers of TT genotype. The distribution of carriers of genotypes taking into account incorrect posture showed that it was significantly more often found in carriers of two genotypes of homozygous TT (p = 0.0001) and heterozygous CT (p = 0.0005, Table 4).

Regarding the polymorphism of the gene intracellular vitamin D receptor VDR , it was found that the TT genotype is associated with a tendency to delay growth rate associated with impaired bone metabolism and osteosynthesis during activation of bone resorption [19]. When studying the distribution of rs731236 TaqI VDR alleles: c.1056T> C, we found that carriers of the C allele made up 94% of the subjects (Table 5). The homozygous

Table 1

Parameters of the state of the musculoskeletal system in teenagers

Age	15,607 (15;17)
Gender	Man - 38.4% Woman - 61.6%
Health group	1 – 1,8 %; 2 – 98,2 %
Body length, cm	163,4 (140;180)
Body weight, kg	60 (40;80)
Body mass index	22,4 (16;30)
Types of posture	without violations - 40.2%; kyphosis - 15.2%; lordosis - 3.6%; straightened - 42%
Body type	normosthenic - 79.5%; hypersthenic - 8.5%; asthenic - 12%
Scoliosis, classification by V.D. Chaklin	no - 63.3%; 1 degree - 34%; 2 degree - 2.7%
Leg shape	correct - 68.9%; X-shaped - 30.1%; O shaped - 1%
Valgus / Varus	valgus – 7,1 %; varus – 1,8 %
Hallux valgus valgus deformity (Hallux valgus)	S – 66,1 %; D – 60,7 %
Flat feet - transverse - longitudinal	 1 degree - 33%; 2 degree - 54.5%; grade 3 - 2.7% 1st degree - 28.6%; 2nd degree - 7.1%
Pelvic bone displacement	no - 82.2%; right - 8.8%; left - 8.8%
Joint hypermobility	no - 79.8%; availability - 20.2%
Fractures,% depending on the amount: 0-no; one; 2; 3 and more	0 – 67,9 %; 1 – 25,9 %; 2 – 4,5 %; 3 – 1,8%
Dislocations:% depending on the amount: 0-no; one; 2; 3 and more	0 – 88,4 %; 1 – 9,8 %; 2 – 1,8 %

TT genotype of the TaqI VDR gene was more common among girls than among boys (19.6% and 3.9%, respectively), with a predominance of 5 times (Table 2). Moreover, in carriers of heterozygous CT and homozygous TT genotypes, the presence of fractures was more often noted (p = 0.09, Table 3; p = 0.01, Table 4).

According to the researchers, the carriage of the polymorphism variants genes type I collagen (COL1A1 (rs1800012)), calcitonin receptors (CALCR 1340 (rs1801197)) and vitamin D (TaqI VDR (rs 731236)) is associated with the development of diseases associated with a rapid loss of bone mineral density tissue and the development of osteoporosis [2, 11, 15-17, 20, 23, 24, 25]. There was a correlation between the gene polymorphism calcitonin receptor C1377T and a decrease in bone mineral density in the lumbar spine in the postmenopausal period in women [12]. Also a direct relationship between the TaqI rs731236 variant of the VDR gene the allele TT and the presence of osteoporosis, and heterozygous CT genotype more common in women with fractures [4]. The results of our study revealed that from 3.8% to 50% of the examined had such combinations in various variants, and a combination of two “unfavorable” alleles was found

Table 2

The genotype distribution in examined teenagers

Collagen gene rs1800012 COL1A1:c.104–441G>T, (n=88)			
Genotype options	SS (GG)	ss (TT)	Ss (GT)
Male	2.0	0	37.3
Female	7.7	0	53.0
Gene of calcitonin receptor rs 1801197 CALCR:c.1377C>T (n=88)			
Genotype options	CC	TT	CT
Male	8	2.0	29.0
Female	6	4.0	51.0
Gene of intracellular vitamin receptor D rs731236 TaqI VDR:c.1056T>C (n=88)			
Male	3.9	3.9	31.4
Female	5.9	19.6	35.3

Примечание. В табл. 3 и 4 различие статистически значимо при p<0,5.

Table 3

Table 3. Correlation coefficient between the presence of fractures and variants of genotypes

Genotypes	N	Fisher coefficient	
		Empirical	Critical, α=0.05
COL1A1:c,104–441G>T			
SS (GG)	88	0,79; p=0,072	1,31
Ss (GT)	88	2,47; p=0,123*	
rs 1801197 CALCR:c,1377C>T			
CC	88	1,63; p=0,211	1,31
TT	88	2,125; p=0,141*	
CT	88	0,36; p=0,851	
rs731236 TaqI VDR:c,1056T>C (n=88)			
CC	88	0,52; p=0,473	1,31
TT	88	0,53; p=0,471	
CT	88	2,88; p=0,091*	

in 46.15%: the G allele of type I collagen polymorphism (rs1800012 COL1A1) and the T allele TaqI polymorphism of the vitamin D receptor gene, and 33.3% of the examined had a combination of 3 "predisposition" alleles at once (Table 5).

Our comprehensive assessment of the MSS health status in teenagers of the Primorsky Territory showed a harmonious development in 109 (47.3%) of 200 people in the surveyed group. Mostly, teenagers have a second health group. Kyphotic, hyperlordosis and hyperkyphotic posture and the presence of lateral curvature of the spine were more often observed in girls than in boys, and photoplantography showed the presence of normal foot development in only 9% of the subjects. Our data demonstrate the need to study the physiological parameters of the teenagers musculoskeletal system state, since it is known that in children suffering from flat feet and scoliosis, there is a violation of blood circulation and the development of concomitant diseases [6, 5]. Such studies will allow the implementation of preventive measures aimed at preventing the development of diseases.

Simultaneously, we conducted genotyping of study participants for the presence of gene variants single-nucleotide substitutions for rs1800012 IVS1 c.2046 G> T collagen COL1A1 rs1801197 c.1377 T> C calcitonin receptor CALCR and rs731236 c.1056 T> C intracellular vitamin D receptor VDR. In the examined cohort of the teenagers Primorsky Territory, carriers of the heterozygous Ss (GT) genotypes (87.3%, the collagen gene COL1A1) and CT (80% - the calcitonin receptor gene and 66, 7% - the intracellular vitamin D receptor gene, respectively) are predominant. In carriers of the heterozygous Ss (GT) COL1A1 gene, the presence of the heterozygous CT genotype of calcitonin receptor genes (CALCR) and vitamin D VDR was more common. The genetic testing also revealed a relationship between the frequency of genes variants distribution that regulate the mineralization process in teenagers and the presence of fractures and impaired posture (from 6.1 to 79.2%). The interest is the revealed relationship between the homozygous genotypes TT of the studied gene polymorphisms calcitonin receptor and the heterozygous CT gene of the intracellular vitamin D receptor VDR with the presence of breaks and impaired posture, especially in girls. In general, the results indicate a combined effect of genes on mineral metabolism in the body, which begins to manifest itself in the teenage period at the phenotypic level. In our opinion, special attention should be paid to the subjects with a combination of several "unfavorable" gene polymorphisms, since it is for them that the risk of metabolic disturbance is highest.

Currently, there is numerous evidence that under the influence of "variable" factors acting in prenatal, childhood or adolescence, the programmed peak bone mass decreases, which can cause the development of not only a juvenile form of systemic osteoporosis, but also its postmenopausal and senile forms [4, 12]. It has been demonstrated that the presence of fractures in parents (in particular, a hip fracture) increases the risk of fractures regardless of the bone mineral density [13]. Carriage of unfavorable alleles in the genes of type 1 collagen (rs 1800012 Col1A1), G-1997 (rs 1107946 Col1A1), calcitonin receptor (rs1801197 CALCR) and vitamin D (TaqI rs 731236 VDR) is connected with the development of diseases associated with loss of bone mineral density and the development of osteoporosis [7, 16, 24]. It has also been shown that, in carriers of the collagen COL1A1 heterozygous genotype, fractures and impaired posture are more common, which is consistent with other studies of the relationship between the presence of

Table 4

Table 4. Distribution of the genotypes of the subjects (%) depending on the presence of fractures, flat feet and impaired posture

	COL1A1:c,104–441G>T		rs 1801197 CALCR:c,1377C>T			rs731236 TaqI VDR:c,1056T>C		
	SS (GG)	sS (GT)	CC	TT	CT	CC	TT	CT
The presence of fractures (N=27)	23.1	53.85	3.85	15.4	57.7	7.7	23.1	46.2
No fractures (N=61)	22.95	16.4	3.28	27.7	8.23	8.2	1.6	29.5
t-criterion emp.	2.01 *p=0.561*	2.0* *p=0.073*	1.99 *p=0.111*	2.0 *p=0.984*	2.01* *p=0.062*	2.0 *p=0.431*	2.02* *p=0.054*	2.0* *p=0.012*
Flat feet, longitudinal (N=36)	19.4	69.4	13.9	30.6	47.2	8.3	22.2	61.1
No flat feet detected (N=78)	23.1	65.4	10.3	24.3	55.1	11.5	18.0	60.3
t-criterion emp.	1.99 *p=0.662*	2.0 *p=0.674*	2.0 *p=0.621*	2.0 *p=0.655*	2.0 *p=0.654*	2.0 *p=0.653*	2.0 *p=0.612*	2.0 *p=0.933*
Impaired posture (N=48)	31.25	18.75	4.2	39.6	6.25	10.4	2.1	37.5
No impaired (N=37)	8.1	56.7	5.4	2.7	56.75	8.1	18.9	37.8
t-criterion emp.	1.99* *p=0.0008*	1.99* *p=0.0003*	1.99 *p=0.95*	1.99* *p=0.0001*	1.98* *p=0.0005*	1.98 *p=0.354*	2.0 *p=0.182*	2.0 *p=0.931*

the T allele and bone strength in school-aged children [14]. Thus, in the presence of certain risk factors and timely diagnosis of early preclinical stages of osteoporosis, it is possible to prevent life-threatening complications such as fractures of the vertebral bodies and femoral neck. At the same time, testing of allelic variants of these genes opens up great opportunities for the prevention of bone system pathology, as it allows one to effectively identify individuals with a high risk of disease long before signs of pathological changes in the musculoskeletal system appear and is an area of preventive medicine research.

Conclusions. Thus, genetic testing made it possible, firstly, to identify the prevalence of "unfavorable" alleles and their combinations in the studied genes (from 3.8% to 50%) that regulate calcium metabolism in teenagers; secondly, to determine the relationship between genotypes and the presence of fractures and impaired posture, which makes it possible to distinguish a group of subjects with a high risk of developing MSS diseases. In our opinion, the special attention require from pediatricians the adolescence of children, which is characterized not only by a sharp change in the hormonal status of the organism, but also by a maximum increase in the growth of tubular bones and vertebrae with an increase in the mineral density of bone tissue and muscle mass. During this period, the influence of various exogenous and endogenous stimuli on the formation of peak bone mass and, possibly, the risk of developing osteopenic syndrome is decisive. The data of the analysis confirm the need for the introduction of dynamic studies of the health indicators of the MSS in the socio-hygienic monitoring of children's health as an indispensable element. Despite the small sample size of our study, its results make it possible to assess the contribution of genetic polymorphisms to the development of MSS, which in the future will make it possible to use such data as predictive indicators for organizing personalized prevention programs.

Compliance with ethical standards. *The study protocol was approved by the independent interdisciplinary ethics committee of the Pacific State Medical University (protocol No. 2 dated 10.24.2016).*

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Conflict of interest. *The authors confirmed that there is no conflict of interest to report.*

Table 5

Table 5. Distribution of polymorphisms and alleles of genes in examined adolescents and a combination of the genes variants

Polymorphisms		Distribution in % (n=88)
rs1800012 COL1A1:c.104–441 G>T	SS (GG) Ss (GT) ss (TT) S	9.7 90.3 0 100
rs 1801197 CALCR:c.1377 C>T	CC TT CT C T	11.5 29.5 59 44.4 55.6
rs731236 TaqI VDR:c.1056 T>C	CC TT CT C T	12.8 20.5 66.7 56.1 43.9
The combination of variants of the studied genes	**Genotypes, %**	
Ss + CT (rs1800012 COL1A1:c,104–441; rs731236 TaqI VDR:c,1056)	46.15	
Ss + CT (rs1800012 COL1A1:c,104–441; rs 1801197 CALCR:c,1377)	50	
CT + CT (rs731236 TaqI VDR:c,1056; rs 1801197 CALCR:c,1377)	37.2	
TT + TT (rs731236 TaqI VDR:c,1056; rs 1801197 CALCR:c,1377)	3.8	
Ss + CT + CT (rs1800012 COL1A1:c,104–441; rs731236 TaqI VDR:c,1056; rs 1801197 CALCR:c,1377)	33.3	

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