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Prevalence of Bsml polymorphism (rs1544410) of the VDR gene's genotypic and allelic variants in patients with autoimmune thyroiditis.

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ABSTRACT

BACKGROUND: The most common among AITD is autoimmune thyroiditis (AIT), also known as Hashimoto's thyroiditis. This disease arises from a complex interaction of genetic predisposition and environmental factors.
OBJECTIVE: To assess the impact of allelic and genotypic variants of the VDR gene polymorphism BsmI (rs1544410) on thyroid function in patients with autoimmune thyroiditis.
MATERIALS AND METHODS: The study included 139 patients diagnosed with autoimmune thyroiditis, aged 20 to 44 years, who sought consultative care at the outpatient clinic of the 3rd clinic of Tashkent Medical Academy. Among them, 109 (78.4%) were women with an average age of 34.3±6.4 years, and 30 (21.6%) were men with an average age of 36.8±5.6 years. The control group consisted of 47 individuals with a mean age of 35.6±4.4 years. Serum concentrations of vitamin D – 25(OH)D, anti-thyroglobulin antibodies, anti-thyroid peroxidase antibodies, free thyroxine, free triiodothyronine, and thyroid-stimulating hormone were assessed.
RESULTS: In our study, a trend can be observed that the A allele has a predisposing effect on the development of autoimmune thyroiditis, reaching statistical significance ($\chi2$=19.8; p=0.01), particularly in the development of euthyroidism, subclinical, and manifest hypothyroidism. As for the G allele, statistical data ($\chi2$=19.8; p=0.01) indicate a protective effect, meaning that the G allele has a protective role against autoimmune thyroiditis, particularly in the development of euthyroidism, subclinical, and manifest hypothyroidism.
CONCLUSION: The study results indicated a potential role of the heterozygous A/G genotype in the development of autoimmune thyroiditis. The presence of this genotype significantly increased the risk of the disease by 6.6 times ($\chi2$=11.5; p=0.01; RR=4.9; 95% CI: 3.76-6.28; OR=6.6; 95% CI: 2.22-19.64). Additionally, the presence of the heterozygous A/G genotype increased the risk of autoimmune thyroiditis with euthyroidism more than sixfold and the risk of developing subclinical hypothyroidism more than eightfold. Moreover, the most functionally favorable G/G genotype was more common in the control group, indicating a low probability of its association with the studied disease ($\chi2$=17.9; p=0.01).

Thyroid gland's autoimmune diseases (AITD) are a common pathology affecting up to 5% of the entire population, and their prevalence continues to increase [1,14,17]. The most common among AITD is autoimmune thyroiditis (AIT), also known as Hashimoto's thyroiditis. This disease arises from a complex interaction of genetic predisposition and environmental factors [13]. Thyroid function can be negatively affected by external effects as the intake of certain medications, viral infections, iodine consumption, and radiation exposure, with toxic damage to thyrocytes, provoking the immune system, or activating other immune reactions [1,15]. However, because of genetic

influences, to link directly these external factors to AIT is not simple. Single nucleotide polymorphisms (SNPs) in genes association with AIT was demonstrated in the research and says that this connection may be related to individual susceptibility to this disease [2,15,20].

Increased levels of serum thyroglobulin (Tg) and/or autoantibodies to thyroid peroxidase (TPO), significant lymphocytic infiltration of thyroid tissues, as well as possible atrophy of the gland or goiter can be indicators of AIT. The clinical symptoms of AIT may vary from euthyroidism to subclinical or overt hypothyroidism [13,16]. In euthyroidism, along with the presence of antibodies to TPO and Tg, the levels of thyroid-stimulating hormone (TSH), T3, and T4 remain within the normal range [13].

Several problems with can be associated with deficiency of Vitamin D, including type 1 diabetes, cardiovascular diseases, autoimmune disorders, certain types of cancer, such as breast cancer, allergic reactions and depression [22]. It is believed that Vitamin D exerts effects to the organism through non-genomic and genomic mechanisms [21].

Immunomodulatory properties the active form of vitamin D, carries out its functions by binding to VDR, which affects the activity of immune cells and triggers both innate and adaptive immune responses [3,4,18]. The VDR gene, located on chromosome 12, consists of several exons and is known to contain a number of polymorphisms, the most studied of which are FokI (rs2228570), BsmI (rs1544410), TaqI (rs731236), and ApaI (rs7975232) [18]. The FokI polymorphism is located in the initiation gene's codon, while the BsmI, ApaI, and TaqI polymorphisms are located in the 3' region [19]. It is likely that a combination of genetic and environmental factors contributes to the development and clinical variability of AIT [5,18].

A number of studies [6,9,8,10,11] have shown that the concentration of vitamin D in the serum of patients with AIT is lower than that of healthy individuals. This inverse relationship suggests that a deficiency or insufficiency of cholecalciferol may contribute to the development of AIT and may also influence the course of this disease. In this regard, the level of vitamin D should be considered when studying the relationship between VDR and the risk of developing AIT. However, there is an alternative view that the reduction in vitamin D levels in patients is a consequence of impaired VDR function rather than the underlying cause of autoimmune processes [19]. Although vitamin D levels are considered important, VDR dysfunction is proposed as a key factor in the development of autoimmune diseases [7,13]. As long as in innate of immune response main role played by VDR, it shows the VDR how necessary in autoimmune diseases's pathogenesis [7], so that innate immune respons can be weakened if VDR is dysregulated.

Particular attention is given to the BsmI polymorphism of the VDR gene, which has been the subject of numerous studies demonstrating its potential association with various autoimmune diseases [18,19].

Objective of the study. To analyze the BsmI (rs1544410) polymorphism of the VDR gene on patients with autoimmune thyroiditis, effected by different variants genotypes and allels, taking into account the functional state of the thyroid gland.

Materials and Methods. The study included 139 patients aged 20 to 45 years diagnosed with autoimmune thyroiditis. The study group consisted of 109 women (78.4%) with an average age of 34.3±6.4 years and 30 men (21.6%) with an average age of 36.8±5.6

years. The control group consisted of 47 individuals with an average age of 35.6±4.4 years. All patients underwent assessment of thyroid status and immunological markers.

The final sample included participants residing in the studied area and belonging to the local Uzbek population. Levels of hormones and antibodies were determined using the immunochemiluminescent assay (ICLA) method with a YHLO analyzer (China).

In serum, the concentrations of thyroid-stimulating hormone (TSH), free triiodothyronine (f. T3), free thyroxine (f. T4), antibodies to thyroid peroxidase (anti-TPO), antibodies to thyroglobulin (anti-Tg), as well as the level of vitamin D – 25(OH)D were measured. Additionally, total calcium in the blood was tested using assay kits from HUMAN (Germany).

The reference values used were the norms proposed by the manufacturer of the respective commercial test kits: TSH – 0.3–4.0 mU/L, f. T3 – 2.0–4.2 pg/mL, f. T4 – 8.9–17.2 pg/mL, anti-TPO – 0–30 IU/mL, anti-Tg – 0–100 IU/mL.

The study of the level of 25(OH)D in serum was conducted using the immunochemiluminescent assay (ICLA) method according to the criteria of the International Society of Endocrinology (2011). A value of 25(OH)D above 30 ng/mL was considered normal.

The combination of elevated TSH levels with normal T4 values was regarded as subclinical hypothyroidism, while its combination with lowered T4 levels was considered overt hypothyroidism.

Ultrasound examination (US) of the thyroid gland (TG) was performed using a SonoScape SSI-6000 device (China) with a 7.5 MHz probe. The obtained data on its volume were analyzed according to the norms established in relation to body surface area. The diagnosis of goiter was made when the upper limit of normal values was exceeded.

Genetic testing for the BsmI (rs1544410) polymorphism of the VDR gene was conducted in the molecular genetics laboratory of the Genotechnology clinic.

Statistical processing of the obtained data was conducted using the software 'STATISTICA 10.0' depending on the type of data and the size of the study groups. Mathematical statistical methods were applied in the analysis of clinical material. In particular: frequency analysis (%); methods of variance statistics (mean arithmetic (M), standard deviation (σ), standard error (m), etc.); variance analysis (t-test); correlation analysis (Pearson correlation coefficient r).

The statistical processing of genetic data was performed using the universal statistical software application Epi Info 7.2.2.2, designed for scientific research in the field of epidemiology. Frequency analysis methods, including ROC, AUC, OR, and PHW analyses, were applied in the analysis of genetic material. The criterion for the statistical significance of the obtained conclusions was set at the generally accepted medical threshold of $p < 0.05$.

Results of the Study. The diagnosis of AIT was established based on standard criteria, using functional tests of thyroid stimulating hormone to define thyroid function (TSH), antithyroid antibodies (anti-TPO, anti-Tg) levels were elevated, characteristic ultrasound signs were mentioned either. In the control group, patients did not have thyroid pathology, it was confirmed by history of patient, laboratory indicators (TSH, anti-TPO, anti-Tg) and ultrasound test.

Anthropometric and biochemical characteristics of all subjects—both the control and AIT groups—are summarized in Table 1. A comparison of the primary group with the control group was conducted.

Patients with AIT and the control group were well matched in terms of age and gender. Furthermore, individuals with AIT had significantly elevated levels of TSH, anti-TPO, and anti-Tg, as well as reduced levels of free T3 and free T4 ($p < 0.05$). The levels of 25(OH)D in the blood were lower in both the control and study groups; however, this indicator was 50% lower in the patient group ($p < 0.05$).

Table 1. Clinical and laboratory characteristics of patients with AIT and the control group.

Variables	Control (n = 47)	Patients with AIT (n = 139)	P-value
Age (years); median (interquartile range)	35,6 (30-–43)	35,8 (28–46)	0,066
Gender			
Male (%)	7 (14,8%)	15 (10,3%)	-
Female (%)	40 (85,2%)	130 (89,7%)	-
BMI	28,6±3,2	30,5±2,9	-
AIT in history(%)	–	57 (39,3%)	-
Thyroid gland volume (ml)	11.39±1,2	10.39±1,3	-
TSH (μIU/mL)	2,5±2,2	6,8±1,0	<0,05
Free T3 (pg/ml)	3,1±0,8	1,9±0,02	<0,05
Free T4 (pg/ml)	15,4±1,6	9,3±0,9	<0,05
Anti-TPO (IU/ml)	12,4±2,2	290,5±9,8	<0,05
Anti-TG (IU/ml)	30,2±5,2	621,6±12,4	<0,05
25(OH)D (ng/ml)	26,7±5,7	11,5±2,2	<0,05
Total calcium (mmol/l)	2,3±0,73	2,25±0,54	0,066

Note: significant result in relation to the control group, statistical significance level - $p < 0.05$.

During the ultrasound examination of the thyroid gland, its volume averaged 24.7±3.4 cm^3 ($p<0.05$) in the study group, while in the control group it was 13.5±2.5 cm^3. Comparing the average volumes of the thyroid gland between the control group and the study group revealed the following: the average volume of the thyroid gland was 13±2.5 cm^3 (control), 11±3.0 cm^3 in the group with mild goiter (MG), 24.9±7.2 cm^3 in the group with severe goiter (SG) ($p<0.05$), and 23.3±5.9 cm^3 in the euthyroid group ($p<0.05$). Thus, in the SG and euthyroid groups, the volume of the thyroid gland was higher by 48% and 35%, respectively, compared to the MG group. In this study, 45% exhibited hypertrophic, 25% had atrophic, and 30% had diffuse-nodular forms of AIT.

In our study, the analysis of associations of the rs1544410 polymorphism in the VDR gene was conducted by comparing two samples (patients and conditionally healthy individuals) using a case-control model. Frequencies which are expected and observed illustrated correspondens of the distribution of alleles and genotypes of the BsmI polymorphism in the VDR gene to the theoretically expected distribution under Hardy-Weinberg equilibrium ($p > 0.05$). This

indicates that there are no significant deviations from the expected distribution in the studied population. In the study of conditionally healthy individuals, no homozygous pathogenic genotype A/A was found; this genotype was less prevalent in this population (Table 2).

Table 2. Frequency distribution of alleles and genotypes of the G/A (rs1544410) polymorphism in the VDR gene (BsmI) in patient and control groups.

Num	Group	Allele frequency				Genotype frequency distribution					
		A		G		A/A		A/G		G/G	
		n	%	n	%	n	%	n	%	n	%
1	Main group (n = 139)	66	23,79	212	76,21	12	8,28	43	31,03	84	60,69
3	Euthyroidism (n = 77)	37	24,03	117	75,97	7	9,09	23	29,87	47	61,04
4	Subclinical hypothyroidism (n = 42)	23	27,38	61	72,62	4	9,52	15	35,71	23	54,76
5	Manifest hypothyroidism (n = 20)	6	15	34	85	1	5	4	20	15	75
6	Control group (n = 47)	3	3,19	9	96,81	0	0	3	6,38	44	93,62

As a result of studying the frequency of allelic variants of the BsmI polymorphism in the VDR gene, it was found that allele A was significantly more frequent in the main group than in the comparison group, with a frequency of 23.8% versus 3.2%, respectively. ($\chi2$=19.8; p=0.01; RR=7.5; 95%CI:6.3-8.82; OR=9.5; 95%CI:3.52-25.51). The statistical analysis of allele A ($\chi2$=19.8; p=0.01) confirms the significance of the differences, and the calculated relative risks and odds indicate a high probability of this allele's influence on disease susceptibility (tables 1, 2, figure 1).

Conversely, allele G was significantly more prevalent in the control group compared to the main group, with a frequency of 96.8% versus 76.2%, respectively ($\chi2$=19.8; p=0.01; RR=0.1; 95%CI:0.01-1.21; OR=0.1; 95%CI:0.04-0.28), indicating its protective role. Statistical analysis ($\chi2$=19.8; p=0.01) confirms the significance of the differences, and the calculated odds ratio (OR=0.1) indicates a low probability of association of this allele with the studied disease. These results highlight the importance of allele G as a potential protective factor (tables 2, 3, figure 1).

Table 3. Differences in the frequency of allelic and genotypic variants of the G/A (rs1544410) polymorphism in the VDR gene (BsmI) in patient groups.

Alleles and genotypes	Number of alleles and genotypes				χ2	p	RR	95%CI	OR	95%CI
	Main group		Control group							
	n	%	n	%						
A	66	23,8	3	3,2	19,8	0,01	7,5	6,3-8,82	9,5	3,52-25,51
G	212	76,2	91	96,8	19,8	0,01	0,1	0,01-1,21	0,1	0,04-0,28
A/G	43	31,0	3	6,4	11,5	0,01	4,9	3,76-6,28	6,6	2,22-19,64
G/G	84	60,7	44	93,6	17,9	0,01	0,6	0,5-0,84	0,1	0,04-0,3

The frequency distribution of the A/G and G/G genotypes was 31.0% and 60.7% (in the control group, 6.4% and 93.6%), and the heterozygous A/G genotype was also more frequent at 31.0% versus 6.4%, respectively ($\chi2$=11.5; p=0.01; RR=4.9; 95%CI:3.76-6.28; OR=6.6; 95%CI:2.22-19.64). Statistical analysis ($\chi2$=11.5; p=0.01) confirms the significance of the differences, and the calculated relative risks (RR=4.9) and odds (OR=6.6) indicate an increased likelihood of this genotype being present in the main group. These results emphasize the potential role of the heterozygous A/G genotype in disease predisposition (tables 1, 2, figure 1).

The frequency of the homozygous G/G genotype was significantly higher, at 93.6% compared to 60.7%, respectively. ($\chi2$=17.9; p=0.01; RR=0.6; 95%CI:0.5-0.84; OR=0.1; 95%CI:0.04-0.3), indicating its protective role. Statistical analysis ($\chi2$=17.9; p=0.01) confirms the significance of the differences, and the calculated relative risks (RR=0.6) and odds (OR=0.1) indicate a low probability of association of this genotype with the studied disease. These results highlight the importance of the homozygous G/G genotype as a potential protective factor (tables 1, 2, figure 1).

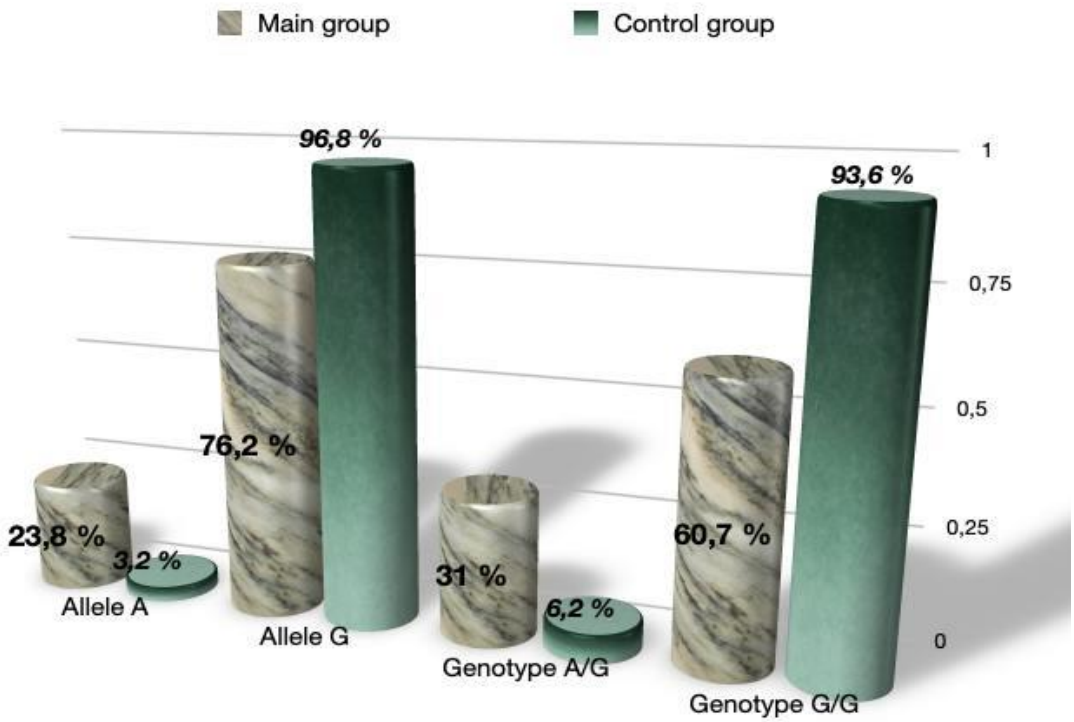

Figure 1. Frequency of allelic and genotypic variants of the G/A (Bsml) polymorphism in the VDR gene in patient groups

Table 4. Differences in the frequency of allelic and genotypic variants of the G/A (rs1544410) polymorphism in the VDR gene (BsmI) in the euthyroid patient group and the control group.

Alleles and genotypes	Number of alleles and genotypes				χ2	p	RR	95%CI	OR	95%CI
	Euthyroidism		Control group							
	n	%	n	%						
A	37	24,0	3	3,2	18,7	0,01	7,5	5,62-10,08	9,6	3,45-26,71
G	117	76,0	91	96,8	18,7	0,01	0,1	0,02-1,15	0,1	0,04-0,29
A/G	23	29,9	3	6,4	9,7	0,01	4,7	3-7,29	6,2	1,97-19,77
G/G	47	61,0	44	93,6	15,9	0,01	0,7	0,42-1,02	0,1	0,04-0,32

The frequency distribution of alleles in the BsmI polymorphism in the VDR gene showed that allele A frequency is notable more in the group of euthyroidism patients compared to the control group, with a frequency of 24.0% versus 3.2%, respectively (χ2=18.7; p=0.01; RR=7.5; 95%CI:5.62-10.08; OR=9.6; 95%CI:3.45-26.71). The statistical values of χ^2 (18.7) and p (0.01) indicate the significance of the differences, suggesting a low probability of random distribution. The risk ratio (RR=7.5; 95% CI: 5.62-10.08) and odds ratio (OR=9.6; 95% CI: 3.45-26.71) confirm that high risk of developing the disease in the euthyroid form compared to the control group ssociated with the presence of allele A (tables 2, 4, figure 2).

In the control group, allele G was significantly more frequent than in the euthyroid patient group, with a frequency of 96.8% versus 76.0%, respectively (χ2=18.7; p=0.01; RR=0.1; 95%CI:0.02-1.15; OR=0.1; 95%CI:0.04-0.29). The values of χ^2 (18.7) and p (0.01) confirm the statistical significance of the differences. The odds ratio (OR=0.1; 95% CI: 0.04-0.29) indicates that the presence of allele G is associated with a significantly lower risk of disease progression in euthyroidism. These data confirm that allele G may play a protective role (tables 2, 4, figure 2).

The statistical indicators confirm the significance of the differences, with a risk ratio (RR=4.7; 95% CI: 3-7.29) and odds ratio (OR=6.2; 95% CI: 1.97-19.77) suggesting that the presence of this genotype may be associated with potential predisposition to thyroid diseases, even in patients who do not exhibit obvious symptoms (tables 2, 4, figure 2).

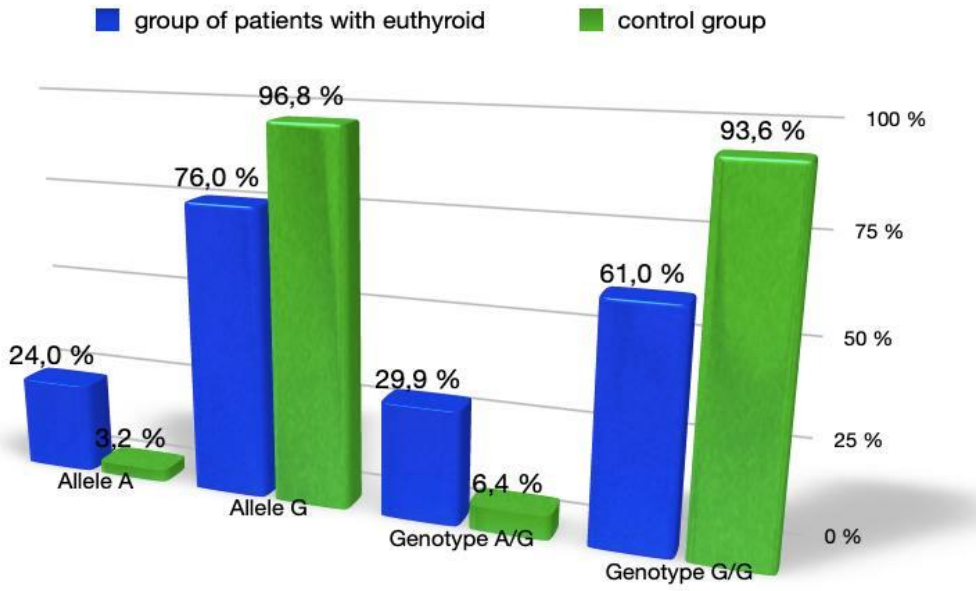

Figure 2. The frequency of allelic and genotypic variants of the G/A polymorphism in VDR gene in group of patients with euthyroid and the control group

The frequency of the homozygous G/G genotype was significantly higher in the control group compared to the group of patients with euthyroidism, consisting of 93.6% versus 61.0%, respectively (χ2=15.9; p=0.01; RR=0.7; 95%CI:0.42-

1.02; OR=0.1; 95%CI:0.04-0.32). The statistical data confirm the significance of the differences, with the risk ratio (RR=0.7; 95% CI: 0.42-1.02) and odds ratio (OR=0.1; 95% CI: 0.04-0.32) indicating that the presence of the homozygous G/G genotype is associated with a significantly lower risk of developing euthyroidism compared to the studied group. These results may suggest a potential protective role of the G/G genotype in relation to the development of the disease (tables 2, 4, figure 2).

Table 5. Differences in the frequency of allelic and genotypic variants of the G/A (rs1544410) polymorphism in the VDR gene (BsmI) in the group of patients with subclinical hypothyroidism and the control group.

Alleles and genotypes	Number of alleles and genotypes				χ2	p	RR	95%CI	OR	95%CI
	Manifest hypothyroidism		Control group							
	n	%	n	%						
A	23	27,4	3	3,2	20,8	0,01	8,6	5,37 - 13,7	11,4	4,01 - 32,59
G	61	72,6	91	96,8	20,8	0,01	0,1	0,01 - 0,95	0,1	0,03 - 0,25
A/G	15	35,7	3	6,4	11,8	0,01	5,6	2,75 - 11,37	8,1	2,47 - 26,93
G/G	23	54,8	44	93,6	18,0	0,01	0,6	0,28 - 1,21	0,1	0,03 - 0,26

The results showed that allele A was higher in the group of patients with subclinical hypothyroidism, at 27.4%. The analysis VDR gene's alleles in the BsmI polymorphism frequency among patients with subclinical hypothyroidism and in the control group indicated that in comparison to the control group—27.4%, significantly more frequent of allele A in the subclinical hypothyroidism group founded 3.2%, respectively (χ2=20.8; p=0.01; RR=8.6; 95%CI:5.37-13.7; OR=11.4; 95%CI:4.01-32.59). Conversely, allele G was significantly more prevalent in the control group compared to the patient group, with frequencies of 96.8% versus 72.6%, respectively (χ2=20.8; p=0.01; RR=0.1; 95%CI:0.01-0.95; OR=0.1; 95%CI:0.03-0.25). The statistical indicators for the alleles confirm the significance of the differences. The risk ratio and odds ratio suggest that the high risk of subclinical hypothyroidism connected with presence of allele A, in the other side protective role in the context of subclinical hypothyroidism is associated presence of allele G (tables 2, 5, figure 3).

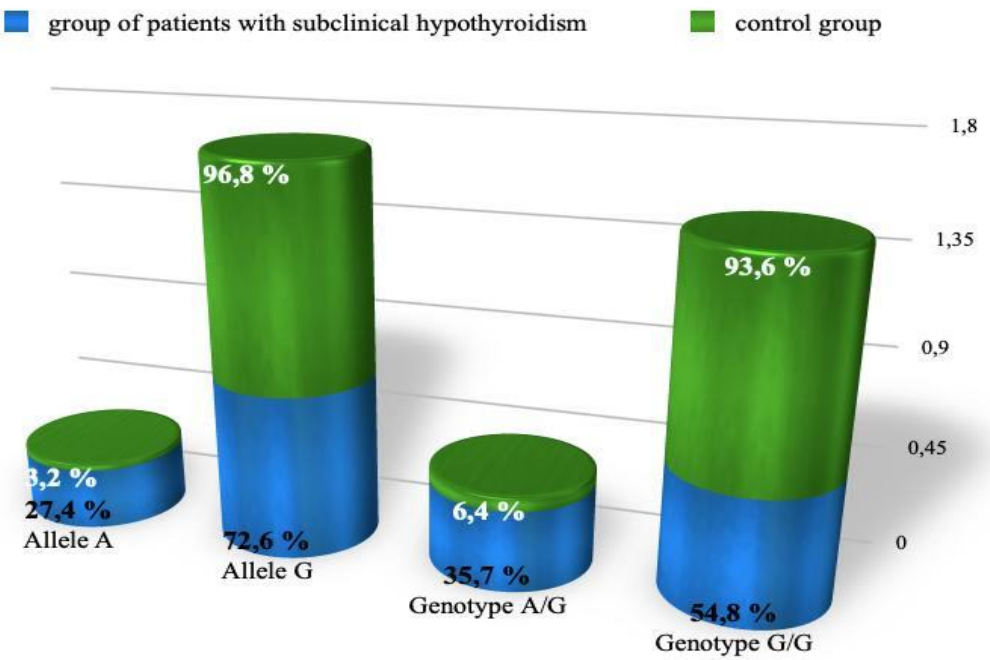

Figure 3. Frequency of allelic and genotypic variants of the G/A polymorphism (rs1544410) in the VDR(BsmI) gene in the group of patients with subclinical hypothyroidism

In patients with subclinical hypothyroidism, the frequencies of the A/G and G/G genotypes of the BsmI polymorphism in the VDR gene were 35.7% and 54.8%, respectively (in the control group, 6.4% and 93.6%). The heterozygous genotype A/G was also significantly more frequent, which may suggest its possible association with the development of this condition ($\chi 2$=11.8; p=0.01; RR=5.6; 95%CI:2.75-11.37; OR=8.1; 95%CI:2.47-26.93). The frequency of the homozygous G/G genotype was significantly higher in the control group compared to the group of patients with subclinical hypothyroidism, with a frequency of 93.6% versus 54.8%, respectively ($\chi 2$=18.0; p=0.01; RR=0.6; 95%CI:0.28-1.21; OR=0.1; 95%CI:0.03-0.26). The statistical indicators confirm the significance of the differences, and the risk ratio and odds ratio indicate that a high risk of developing subclinical hypothyroidism associated with presence of the heterozygous genotype A/G, while potential protective role of this disease the explained with presence of the homozygous G/G genotype (tables 2, 5, figure 3).

Table 6. Differences in the frequency of allelic and genotypic variants of the G/A polymorphism (rs1544410) in the VDR(Bsml) gene in a group of patients with overt hypothyroidism and a control group

Alleles and genotypes	Number of alleles and genotypes				χ2	p	RR	95%CI	OR	95%CI
	Subclinical hypothyroidism		Control group							
	n	%	n	%						
A	6	15,0	3	3,2	6,2	0,03	4,7	1,62 - 13,64	5,4	1,44 - 19,95
G	34	85,0	91	96,8	6,2	0,03	0,2	0,03 - 1,32	0,2	0,05 - 0,7
A/G	4	20,0	3	6,4	2,8	0,10	3,1	0,7 - 14,07	3,7	0,8 - 16,89
G/G	15	75,0	44	93,6	4,6	0,05	0,8	0,21 - 3,11	0,2	0,05 - 0,87

In patients with manifest hypothyroidism, the BsmI polymorphism in the VDR gene was also found to be more frequent, at 15.0% compared to 3.2%, respectively (χ2=6.2; p=0.03; RR=4.7; 95%CI:1.62-13.64; OR=5.4; 95%CI:1.44-19.95). The allele G was significantly more prevalent in the control group compared to the group of patients with manifest hypothyroidism, with frequencies of 96.8% versus 85.0%, respectively (χ2=6.2; p=0.03; RR=0.2; 95%CI:0.03-1.32; OR=0.2; 95%CI:0.05-0.7). The statistical data confirm the significance of the differences in alleles, with the risk ratio and odds ratio indicating that the high risk of manifest hypothyroidism associated with presence of allele A is an while the presence of allele G suggests a protective role regarding manifest hypothyroidism (tables 2, 6, figure 4).

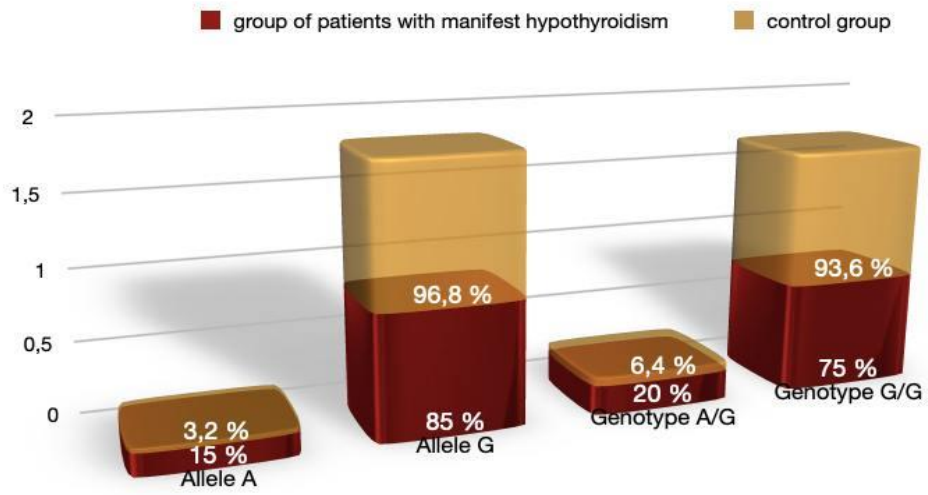

Figure 4. Frequency of allelic and genotypic variants of the G/A polymorphism (rs1544410) in the VDR(BsmI) gene in the group of patients with manifest hypothyroidism

The genotypes A/G and G/G of the BsmI polymorphism in the VDR gene were found in 20.0% and 75.0% of patients, respectively, compared to 6.4% and 93.6% in the control group. Additionally, the frequency of the heterozygous genotype A/G was numerically higher in this group compared to the control group, with frequencies of 20.0% versus 6.4%, respectively ($\chi2$=2.8; p=0.10; RR=3.1; 95%CI:0.7-14.07; OR=3.7; 95%CI:0.8-16.89).

The frequency of the homozygous G/G genotype was significantly higher in the control group compared to the group of patients with manifest hypothyroidism, with frequencies of 93.6% versus 75.0%, respectively ($\chi2$=4.6; p=0.05; RR=0.8; 95%CI:0.21-3.11; OR=0.2; 95%CI:0.05-0.87).

Based on the provided data, a p-value of 0.05 indicates the threshold of statistical significance. The relative risk (RR=0.8) suggests that the event risk in one group is lower, but the wide confidence interval (95% CI: 0.21-3.11) limits confidence in this conclusion. The odds ratio (OR=0.2) shows that the odds of the event in one group are significantly lower, and the confidence interval (95% CI: 0.05-0.87) does not include 1, which confirms the significance of the result. Overall, the data suggest a protective effect but require further research for a more accurate assessment (tables 2, 6, figure 4).

In the study by Djurovic J., Stojkovic O., Ozdemir O., et al., conducted on a case-control basis among Polish patients with autoimmune thyroiditis (AIT), no significant associations were found between the polymorphism rs1544410 of the VDR gene and the risk of developing the disease. However, there are publications indicating a link between VDR polymorphisms and AIT in representatives of the Caucasian race: it has been established that rs2228570 influences the disease risk in Serbian patients, while rs1544410 affects it in Croatians.

Thus, our study noted a significant association of certain alleles and genotypes of the BsmI polymorphism in the VDR gene with the development of autoimmune thyroiditis. In particular, our research

demonstrates a trend indicating that allele A has a predisposing effect on the development of autoimmune thyroiditis, achieving statistical significance ($\chi2$=19.8; p=0.01), especially in the development of euthyroidism, subclinical, and manifest hypothyroidism. Regarding allele G, the statistical data ($\chi2$=19.8; p=0.01) suggest a protective effect of allele G concerning autoimmune thyroiditis, particularly in the development of euthyroidism, subclinical, and manifest hypothyroidism.

Furthermore, the statistical data emphasize the potential role of the heterozygous genotype A/G in the development of autoimmune thyroiditis (AIT). The presence of this genotype increases the risk of developing the disease by 6.6 times ($\chi2$=11.5; p=0.01; RR=4.9; 95%CI:3.76-6.28; OR=6.6; 95%CI:2.22-19.64). With the heterozygous A/G genotype, the risk of AIT with euthyroidism and subclinical hypothyroidism increases by more than 6 and 8 times, respectively. Meanwhile, the most functionally favorable genotype G/G was higher in the control group, indicating a low probability of its association with the studied disease ($\chi2$=17.9; p=0.01).

Based on ROC analysis, the highest values of the area under the curve (AUC) were observed in carriers of the heterozygous A/G genotype, particularly in hyperthyroidism (AUC = 0.72), indicating its moderately good predictive ability for this condition; however, statistical significance was not achieved ($p > 0.05$). The A allele also demonstrated moderate predictive potential (AUC 0.61–0.62), but likewise without statistically significant differences. At the same time, the G allele and the homozygous G/G genotype were associated with low AUC values (0.34–0.44), reflecting their poor diagnostic utility.

Thus, none of the alleles or genotypes under study showed high individual predictive accuracy or statistical significance, which limits their independent application in clinical diagnostics.

The odds ratio assessment suggests a possible association of the A allele and the heterozygous A/G genotype with an increased risk of thyroid disorders, particularly hyperthyroidism and overt hypothyroidism, whereas the G allele and the G/G genotype appear to play a protective role. However, statistical significance was not reached ($p>0.05$), which restricts their clinical applicability.

Conclusion. These results underscore the importance of the homozygous G/G genotype as a potential protective factor regarding euthyroidism and subclinical hypothyroidism. There is a clear pattern indicating that this genotypic variant of the BsmI polymorphism in the VDR gene does indeed exert a protective effect with a high degree of statistical significance. In patients of European descent with AIT, the only significant association was found for the SNP rs1544410.

The authors noted that patients with the BsmI polymorphism more frequently suffered from autoimmune thyroid diseases, which is in agreement with our findings. Thus, the results of other authors confirm the significance of the VDR polymorphism in the development of autoimmune thyroiditis, although the associations observed may vary depending on the population and research methods, highlighting the need for further studies to gain a more comprehensive understanding of the genetic factors influencing disease development.

The odds ratio analysis indicates a potential link between the A allele and the heterozygous A/G genotype with an

elevated risk of thyroid disorders, especially hyperthyroidism and overt hypothyroidism, while the G allele and the homozygous G/G genotype seem to exert a protective effect. Nonetheless, the lack of statistical significance ($p>0.05$) limits their applicability in clinical practice.

Authors' Contribution: U.D.A. conceptualized and designed the study, collected and evaluated the clinical data, performed the statistical analyses, interpreted the results, and drafted the manuscript. U.D.A. critically revised and approved the final version of the manuscript.

Conflict of interest: D.A. reports a relationship with the Tashkent Medical Academy.

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