



Association between Serum Brain-derived Neurotrophic Factor and 25-OH Vitamin D Levels with Vitamin D Receptors Gene Polymorphism (rs2228570) in Patients with Autoimmune Thyroiditis and Hypothyroidism

Iryna Kamyshna^{1*}, Larysa Pavlovych², Aleksandr Kamyshnyi³

¹Department of Medical Rehabilitation, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine; ²Department of Clinical Immunology, Allergology and Endocrinology, HSEEU, Bukovinian State Medical University, Chernivtsi, Ukraine; ³Department of Microbiology, Virology, and Immunology, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

Abstract

BACKGROUND: Different polymorphisms in Vitamin D receptors (VDRs) have an important role in autoimmune thyroiditis (AIT) risk. Hashimoto's thyroiditis (HT) is the most recurrent autoimmune thyroid disorder. Patients with HT may suffer from cognitive impairment brain-derived neurotrophic factor (BDNF) which has been identified as an important growth factor that is involved in learning and memory.

AIM: This study examined the linkage of VDR gene polymorphism (rs2228570) with blood serum levels of BDNF and 25-OH Vitamin D in thyroid pathology of patients in the West Ukrainian population.

METHODS: This research is a case-control study was performed in HSEEU "Bukovinian State Medical University," Chernivtsi Regional Endocrinology Center, and I. Horbachevsky Ternopil National Medical University, Ukraine, from September 2017 to December 2020. The study involved a total of 153 patients with post-operative hypothyroidism, hypothyroidism induced by AIT, and patients with both AIT and elevated serum antibodies anti-thyroglobulin (anti-Tg) and anti-thyroid peroxidase. BDNF levels in the sera of the patients and healthy individuals were quantified using enzyme-linked immunosorbent assay (ELISA) with highly sensitive Human BDNF ELISA Kit. Genotyping of the VDR (rs2228570) gene polymorphism using TaqMan probes and TaqMan Genotyping Master Mix (4371355) on CFX96™ Real-Time Polymerase Chain Reaction (PCR) Detection System (Bio-Rad Laboratories, Inc., USA). PCR for TaqMan genotyping was carried out according to the kit instructions (Applied Biosystems, USA).

RESULTS: Our study revealed a significant decrease in the BDNF level in the study group in carriers of the AA and AG genotypes by 1.58 and 2.39 times, corresponding, compared with carriers of the AA genotype in the control group. Concurrently, there was no significant difference in the BDNF level between different genotypes of VDR rs2228570 in the research group. In our study, analysis of the correlation between serum BDNF levels and 25-OH Vitamin D concentration shows a moderate direct relationship ($r = 0.4$) between BDNF and 25-OH Vitamin D ($p = 0.006$).

CONCLUSIONS: The rs2228570 VDR polymorphism is not a risk factor for decreased serum BDNF levels. At the same time, our study found a moderate direct relationship between serum BDNF levels and 25-OH Vitamin D.

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***Correspondence:** Iryna Kamyshna, Department of Medical Rehabilitation, I. Horbachevsky Ternopil National Medical University; Majdan Voli 1, Ternopil, Ukraine. E-mail: kamyshna_ii@tdmu.edu.ua
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Introduction

Hashimoto's thyroiditis (HT) is the most recurrent autoimmune thyroid disorder (AITDs). It causes chronic inflammation of the thyroid tissue, with a condition of hypothyroidism in about 20–30% of patients [1]. Vitamin D plays a role in AIT, as investigated in the past years [2]. Different polymorphisms in Vitamin D receptors (VDR) have an important role in AIT risk [2]. In recent times, several studies have also investigated the association of the four VDR SNPs and HT susceptibility [3]. A growing body of research suggests that patients with HT may suffer from cognitive impairment [4]. Brain-derived neurotrophic factor (BDNF) has been identified as an important growth

factor that is involved in learning and memory [5]. BDNF is directly regulated by thyroid hormones and has a key role in developing the fetal brain [6]. BDNF is expressed in a variety of cells, including neurons, and can cross the blood-brain barrier [7]. A prior study demonstrated a positive correlation between BDNF levels and the function of the central nervous system and peripheral nervous system [8]. Moreover, BDNF, expressed in the brain, is involved in the development of mental disorders, and studies show that peripheral BDNF can potentially be used as a marker of these diseases in humans [9]. Previously, we demonstrated that autoimmune thyroiditis (AIT) and hypothyroidism could affect the transcription of the genes involved in neurogenesis, nerve impulse transmission, and cell cycle regulation [10], [11], [12], [13], [14]. These changes in gene expression can play a role in the development

of neurological complications associated with thyroid pathology.

The aim was to study the linkage of VDR gene polymorphism (rs2228570) with blood serum levels of BDNF and 25-OH Vitamin D in thyroid pathology of patients in the West Ukrainian population.

Materials and Methods

This research was performed in HSEEU “Bukovinian State Medical University,” Chernivtsi Regional Endocrinology Center, and I. Horbachevsky Ternopil National Medical University, Ukraine, from September 2017 to December 2020. The study involved a total of 153 patients with various forms of thyroid pathology. The subjects were divided into three groups. Group 1 (n = 16) included patients with postoperative hypothyroidism (PO); Group 2 (n = 65) included patients with hypothyroidism induced by AIT, and Group 3 (n = 72) included patients with both AIT and elevated serum antibodies anti-thyroglobulin (anti-Tg) and anti-thyroid peroxidase (anti-TPO). Twenty-five healthy individuals were recruited as a control group, randomly and without matching for age or sex.

Ethical approval

The study fully adhered to principles outlined in the 1975 Helsinki Declaration of Human Rights (amended in 2008). The participants enrolled voluntarily after completing and signing a written informed consent. The approval for this study was obtained from the Ethics Committee of the HSEEU “Bukovinian State Medical University,” I. Horbachevsky Ternopil National Medical University and Chernivtsi Regional Endocrinology Center, Ukraine (approval ID: 11-07.11.2017).

Hypothyroidism was diagnosed as recommended by the American Association of Clinical Endocrinologists 2012. When diagnosing AIT, the corresponding clinical features were taken into account, the results of a sonogram of the thyroid gland (reduced echogenicity) and circulating antibodies to thyroid antigens, anti-TPO, and anti-TG were detected [15].

Blood samples from the patients and control individuals were collected in the morning (8–10 AM) after overnight fasting. For each individual who took part in the study, levels of thyroxine (fT4, normal range 6.0–13.0 pmol/L for males and 7.0–13.5 pmol/L for females), thyroid-stimulating hormone (normal range 0.3–4.0 mIU/mL), anti-TPO (normal range 0–30 IU/mL), and anti-thyroglobulin (anti-TG, normal range 0–65 IU/mL) were determined using STAT FAX303/Plus analyzer (Awareness Technology Inc.,

USA).

Study exclusion criteria were as follows: Less than 18 years of age, malignancy, inflammation due to rheumatic diseases or acute/chronic infection, diabetes mellitus, vascular, chronic hepatic or renal diseases, and pregnancy. Individuals taking any drugs that could interfere with thyroid function were also excluded from the study.

BDNF levels in the sera of the patients and healthy individuals were quantified using enzyme-linked immunosorbent assay with highly sensitive human BDNF enzyme-linked immunosorbent assay (ELISA) Kit (Elabscience[®], United States, Catalog No: E-EL-H0010) on E.I.A. Reader Sirio S (Seac, Italy).

25-OH Vitamin D levels in the serum of the patients and healthy individuals were quantified with ELISA using the 25-OH Vitamin D Total (Vitamin D-Direct) Test System ELISA Kit (Monobind Inc.[®], United States, Product Code: 9425-300) on E.I.A. Reader Sirio S (Seac, Italy).

Genotyping of the VDR (rs2228570) gene polymorphism

DNA isolation

To collect venous blood, we used a sterile Vacutainer and stabilized it with K2EDTA. Total DNA was isolated from peripheral blood using PREP-RAPID-GENETICS DNA Extraction Kit (DNA-TECHNOLOGY, Russian Federation), according to the manufacturer's instructions.

DNA amplification and genotyping

The samples were genotyped using TaqMan probes and TaqMan Genotyping Master Mix (4371355) on CFX96™ Real-Time Polymerase Chain Reaction (PCR) Detection System (Bio-Rad Laboratories, Inc., USA). PCR for TaqMan genotyping was carried out according to the kit instructions (Applied Biosystems, USA). TaqMan Genotyping Master Mix contains DNA polymerase AmpliTaq Gold[®], dNTPs, reference dye ROX™, and buffer ingredients. TaqMan probes are target-specific oligonucleotides with reporter dyes attached to the 5' end of each probe: (VIC[®] dye on the 5' end of the Allele 1 probe and 6FAM™ dye on the 5' end of the Allele 2 probe), and a non-fluorescent quencher (NFQ) the 3' end of the probe. Genomic DNA was amplified in a 10 µL reaction mix containing genomic DNA, forward and reverse primers, fluorescent probes, and TaqMan Genotyping Master Mix. Genotyping of the samples performed on the CFX-Manager™ software using the method of allele discrimination based on the magnitude of relative fluorescence units.

Statistical analysis

To determine the difference between groups, we used the Student’s t-test, ANOVA, Pearson’s χ^2 test, odds ratio test, relative odds ratio test, and equality 0 correlation test. The odds ratio and 95% confidence interval were calculated using binary logistic regression; $p < 0.05$ was considered a statistically significant difference between the two groups (Statsoft statistica v.12.0).

Results

In our present study, we investigated the correlation of a VDR polymorphism at the rs2228570 locus and BDNF serum levels of the patients with various thyroid gland pathologies in the Bukovinian region of Ukraine.

The demographic, clinical, and biochemical characteristics of the participants are presented in Table 1.

Table 1: Demographic, clinical, and biochemical characteristics of study participants

Variable	Control group (n = 25)	Patients with post-operative hypothyroidism (PO, n = 16)	Patients with AIT-induced hypothyroidism (AIT with TPO antibodies hypothyroidism, n = 65)	Patients with AIT and elevated anti-Tg and anti-TPO antibodies (AIT, n = 72)
Age (years)	46.08 ± 14.58	47.30 ± 12.27	46.72 ± 15.49	45.02 ± 13.65
ft4 (pmol/L)	8.91 ± 0.97	3.44 ± 0.31	4.13 ± 0.52	8.51 ± 0.82
TSH (mIU/mL)	2.67 ± 0.52	8.61 ± 0.84	7.09 ± 0.50	2.38 ± 0.62
anti-TPO (IU/mL)	34.04 ± 3.70	36.13 ± 2.78	380.62 ± 73.42	330.36 ± 50.23
anti-TG (IU/mL)	15.32 ± 1.97	15.50 ± 1.90	32.97 ± 4.27	36.38 ± 7.70
Current dose of L-thyroxine (µg/day)	None	110.95 ± 5.25	88.46 ± 1.55	None
25-OH Vitamin D ng/mL	39.2 ± 6.58	20.69 ± 3.09 (p < 0.001)	19.08 ± 3.144 (p < 0.001)	21.48 ± 2.83 (p < 0.001)
BDNF (pg/ml)	1037.8 ± 361.83	310.19 ± 112.84 (p < 0.001)	329.34 ± 312.57 (p < 0.001)	1031.34 ± 385 (p = 0.95)

Data are presented as a mean ± standard deviation. BDNF: Brain-derived neurotrophic factor, TSH: Thyroid-stimulating hormone.

We analyzed BDNF levels in patients with different thyroid pathology depending on the VDR rs2228570 genotype (Table 2).

Our study detected a significant decrease in the level of BDNF in the study group in carriers of AA and AG genotypes in 1.58 and 2.39 times, respectively, compared with carriers of AA genotype of the control group (Table 2).

At the same time, a significant difference in the level of BDNF between different genotypes of VDR rs2228570 in the experimental group was not observed.

When conducting a comparative analysis of BDNF content in carriers of different genotypes, depending on thyroid pathology, the lowest indices were in the group of patients with PO. Thus, in patients with AA genotype in the group of patients with PO, the level of BDNF was significantly lower by 3.44 compared with carriers of AA genotype in the control group and patients with AG and

Table 2: BDNF levels in patients with different thyroid pathology depending on the VDR rs2228570 genotype

rs2228570 (M±m)	AA	AG	GG	P5	P6	P7
Control group, n = 25	1006.82 ± 341.62 (9)	1134.5±357.83 (9)	945.8 ± 439.77 (7)			
Study group, n = 153	637.51 ± 490.75 (59)	474.98 ± q351.38 (64)	480.69 ± 363.58 (30)	0.193	0.3034	0.96
P1	0.038	0.00164	0.081			
PO, n = 16	292.67 ± 127.42 (7)	289.02 ± 75.12 (6)	373.47 ± 139.71 (3)	0.95	0.45	0.45
P1	<0.001	<0.001	0.041			
P2	0.0559	0.171	0.958			
AIT with hypothyroidism, n = 65	351.74 ± 265.58 (20)	434.75 ± 339.24 (33)	363.27 ± 380.11 (12)	0.51	0.95	0.72
P1	0.0014	0.0012	0.056			
P3	<0.001	0.136	0.09			
AIT n = 72	1085.4 ± 425.31 (32)	897.3 ± 355.98 (25)	935.05 ± 237.52 (15)	0.4836	0.539	0.896
P1	0.672	0.391	0.96			
P4	<0.001	0.093	0.135			

Statistical significance of differences: p < 0.05. P1 – p-value between the control group and study groups. P2 – p-value between PO and AIT with hypothyroidism groups. P3 – p-value between AIT and AIT with hypothyroidism groups. P4 – p-value between PO and AIT groups. P5 – p-value between AA and AG genotypes. P6 – p-value between AA and GG genotypes. P7 – p-value between AG and GG genotypes. VDR: Vitamin D receptor.

GG genotypes, the level of BDNF was reduced by 3.92 and 2.53 folds, respectively.

When examining patients with AIT with hypothyroidism, the level of BDNF in carriers of the AA genotype was reduced by 2.86 times compared to the AA genotype carriers in the control group. Among carriers of the AG genotype, the BDNF level was significantly lower by 2.6 times compared with the control. In carriers of the GG genotype of patients with AIT with hypothyroidism, the BDNF content did not significantly differ from the control group.

No significant changes in BDNF levels were observed in patients with AIT compared with the control group in carriers of all three genotypes.

Carriers of AA genotype showed a probable decrease in BDNF levels in patients with AIT with hypothyroidism by 3.1 times compared with carriers of AA genotype in the group of patients with AIT.

In addition, the carriers of AA genotype in the group of patients with PO, the level of BDNF was also significantly reduced by 3.7 times compared with carriers of AA genotype among patients with AIT.

However, in a detailed analysis of the BDNF content between carriers AA, AG, and GG in patients of all three groups, depending on the pathology of the thyroid gland, we did not find a significant difference.

Table 3 provides evidence that the rs2228570 VDR polymorphism is not a risk factor for reduced serum BDNF levels.

Table 3: Polymorphic variants of the VDR gene rs2228570 as risk factors for reduced serum

BDNF levels	RelR	OR	95%CI RR	95%CI OR	p
VDR gene rs2228570					
BDNF level <500					
AA	1.053	1.14	0.6799; 1.63	0.3157; 4.3254	1
AG	1.053	1.14	0.6799; 1.63	0.3157; 4.3254	1
GG	0.9211	0.7048	0.6717; 1.2629	0.1577; 3.336	0.734

RelR (relative risk) – relative risk; OR (odds ratio) – odds ratio; 95% CI RR, OR (confidence interval) – confidence interval of risk (RR), chances (OR). VDR: Vitamin D receptor, BDNF: Brain-derived neurotrophic factor.

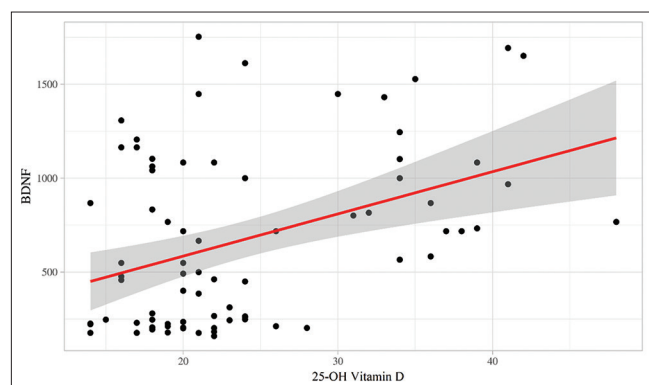


Figure 1: Correlation analysis of the dependence of the "BDNF" indicator on the "Vit D" indicator

An analysis of the correlation between BDNF levels and the levels of 25-OH Vitamin D shows a moderate direct relationship ($r=0.4$) between BDNF and 25-OH Vitamin D ($p=0.006$).

The graph of the regression function, characterizing the dependence of the "BDNF" indicator on the "Vit D" indicator is given in Figure 1. The observed dependence of the "BDNF" indicator on the "Vit D" indicator is described by the equation of paired linear regression:

$$Y_{BDNF} = 135.956 + 22.463 \times X_{Vit D}$$

Where Y BDNF - BDNF,

X Vit D - Vit D.

With an increase in Vitamin D by 1, an increase in BDNF by 22.463 should be expected. By the coefficient of determination R^2 in the resulting model, 15.73% of the factors influencing the value of the "BDNF" indicator were taken into account.

Discussion

BDNF significantly impacts memory formation and brain development, namely, neurogenesis, synaptogenesis, and neuronal differentiation [16]. Previous investigations have detected that thyroid hormones play an essential role in BDNF expression [17]. An essential method used for identifying a hereditary predisposition to certain diseases is based on the analysis of transcriptome [18], [19] and single-nucleotide polymorphism [20], [21], [22].

According to the current evidence, four polymorphisms of the VDR gene (TaqI (rs731236, alleles T/t), Apal (rs7975232, alleles A/a), FokI (rs2228570, alleles F/f), and BsmI (rs1544410, alleles B/b) are associated with the HT risk. However, studies focusing on these polymorphisms have shown discrepant results. A meta-analysis of 11 case-control studies has been conducted intending to establish an association between the four polymorphisms and HT

susceptibility [2].

When conducting a comparative analysis of the BDNF content in carriers of different genotypes of VDR rs2228570, considering the pathology of the thyroid gland, the lowest indices in the group of patients with PO were detected. Consequently, in patients with the AA genotype in the group of patients with PO, the BDNF level was significantly lower by 3.44 compared to carriers of the AA genotype in the control group and patients with the AG and GG genotypes, the BDNF level was reduced by 3.92 and 2, 53 folds correspondingly.

During the examination of patients with AIT with hypothyroidism, the BDNF level in carriers of the AA genotype decreased 2.86 times when compared to carriers of the AA genotype in the control group. Among carriers of the AG genotype, the level of BDNF was significantly lower by 2.6 times compared to the control. In carriers of the GG genotype in AIT patients with hypothyroidism, the BDNF content did not vary notably from the control group.

Our study outcomes show that the rs2228570 VDR polymorphism is not a risk factor for reduced serum BDNF in patients suffering from different forms of thyroid pathology.

According to the late research, an association between low Vitamin D status and autoimmune thyroid diseases such as HT has been demonstrated. Several clinical studies have reported a low Vitamin D status in AITD or HT, showing an association between Vitamin D deficiency and thyroid autoimmunity [23]. Various studies have demonstrated that Vitamin D is crucial for the normal development of the brain and its function, so Vitamin D deficiency has been linked with neurological disorders [24].

The study also detected a decrease in hippocampal BDNF levels in a preclinical model of depression, which made normal with Vitamin D supplementation (Goltz *et al.*, 2017). Vitamin D influences the development and functioning of the brain and the regulation of neurotrophic factors. In our study, analysis of the correlation between serum BDNF levels and 25-OH Vitamin D levels shows a moderate direct relationship ($r = 0.4$) between BDNF and 25-OH Vitamin D ($p = 0.006$).

Conclusions

Our study revealed a significant decrease in the BDNF level in the study group in carriers of the AA and AG genotypes by 1.58 and 2.39 times, corresponding, compared with carriers of the AA genotype in the control group. Concurrently, there was no significant difference in the BDNF level between different genotypes of VDR rs2228570 in the research

group. In addition, the rs2228570 VDR polymorphism is not a risk factor for decreased serum BDNF levels. At the same time, our study found a moderate direct relationship between serum BDNF levels and 25-OH Vitamin D ($p = 0.006$).

It is necessary to conduct ongoing and future long-term randomized controlled trials that will provide insight into the efficacy and safety of Vitamin D as a therapeutic efficacy for these thyroid diseases as the prevention and treatment of neurological complications.

Acknowledgments

Ethical approval

The approval for this study was obtained from the Ethics Committee of the HSEEU "Bukovinian State Medical University" and Chernivtsi Regional Endocrinology Center, Ukraine (approval ID: 11-07.11.2017).

Our study was conducted according to the Declaration of Helsinki adopted in 1975 and revised in 2008, and the ethical principles were entirely respected.

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