OPEN ACCESS This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/ by-nc-nd/4.0), which permits copy and redistribute the material in any medium or format, provided the original work is properly cited.

sciendo

178 ENDOCRINE REGULATIONS, Vol. 56, No. 3, 178–189, 2022

doi:10.2478/enr-2022-0019

Prediction of the cognitive impairment development in patients with autoimmune thyroiditis and hypothyroidism

Iryna I. Kamyshna [1], Larysa B. Pavlovych [2], Aleksandr M. Kamyshnyi [1]

[1]I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine; [2]HSEEU "Bukovinian State Medical University", Chernivtsi, Ukraine
E-mail: Iryna.bilous2017@gmail.com

Objective. The aim of the present work is to define the risk factors that can affect the presence of a cognitive impairment and analyze the associations of the brain-derived neurotrophic factor (BDNF) gene polymorphism (rs6265), vitamin D receptor (VDR) gene polymorphism (rs2228570), and N-methyl-D-aspartate (NMDA) receptor gene polymorphism (rs4880213) with the cognitive impairment in patients with autoimmune thyroiditis and hypothyroidism in the Western Ukraine population and predict the development of cognitive disorders in these patients.

Methods. The study involved 153 patients with various forms of thyroid pathology (hypothyroidism, autoimmune thyroiditis, elevated serum antibodies anti-thyroglobulin and anti-thyroid peroxidase). Cognitive impairment in the examined patients was evaluated based on the results of the Mini-Mental State Examination (MMSE) test. BDNF, GRIN2B, and 25-OH Vitamin D levels in the serum of the patients and healthy individuals were quantified using highly sensitive commercial enzyme-linked immunosorbent assay kits. Genotyping of the VDR (rs2228570), BDNF (rs6265), and NMDA receptor (rs4880213) gene polymorphism was performed using TaqMan probes and TaqMan Genotyping Master Mix (4371355) on CFX96™Real-Time PCR Detection System. Polymerase chain reaction (PCR) for TaqMan genotyping was carried out according to the kit instructions.

Results. Strong direct relationship between the "Level GRIN2B" and cognitive impairments (p=0.006) was established after evaluating the complex model based on the values of the regression coefficient. An increase in the likelihood of cognitive impairment by 24.898-fold (p=0.012) was seen after assessing the effect of the CT rs6265 genotype. In addition, direct relationship between the influence of the TT rs6265 genotype and an increase in the likelihood of cognitive impairment by a factor of 21.734 (p=0.024) was also established.

Conclusion. The present data indicate that the BDNF, TSH, fT4, and vitamin D levels prognostically belong to the significant indicators of the cognitive impairment development.

Key words: autoimmune thyroiditis, BDNF, cognitive impairment, hypothyroidism, vitamin D

The thyroid gland function significantly act upon the human cognitive function and mood. Overt hypothyroidism is accompanied by various disorders affecting neuropsychological and mental activities, namely attention deficit, concentration and memory impairment, psychomotor retardation, depressed mood, and anxiety (Leyhe and Mussig 2014). An increasing number of well-powered studies has reported a cognitive impairment in patients with Hashimoto's thyroiditis (HT), which is not dependent on the thyroid function disorders (Djurovic et al. 2018). Relevant clinical studies have shown a strong

Corresponding author: Iryna I. Kamyshna, MD, PhD, Department of Medical Rehabilitation, I. Horbachevsky Ternopil National Medical University; Majdan Voli 1, Ternopil, Ukraine; phone: +38095431306846001; e-mail: Iryna.bilous2017@gmail.com.

interconnection between vitamin D levels in serum and a cognitive impairment in patients with HT (Xu et al. 2018).

Vitamin D receptors (VDR) and vitamin D-activating enzyme 1α-hydroxylase are located in the hippocampus, hypothalamus, cortex, and subcortex, which are regions vital for cognition (Annweiler et al. 2015). Vitamin D insufficiency has been closely related to the progress of thyrocyte damage in HT and disorders like AITD and HT (Kim 2016).

Brain-derived neurotrophic factor (BDNF), the major member of the neurotrophin family, is broadly expressed in the mammalian brain and engaged in the learning and memory (Sable et al. 2021). Thyroid hormones directly regulate BDNF, which plays an essential role in the brain development (Hung et al. 2018).

There is an increasing need to elucidate the risk factors that may influence the presence of cognitive impairment in patients with HT, since the cognitive impairment is associated with a high risk of depression and disruption of the patient's daily life suffering from chronic diseases (LeMoult and Gotlib 2019). One of the relevant directions of the biomedical research is to investigate the role of genetic factors in the development of common multifactorial diseases (Dzhuryak et al. 2020; Kamyshna and Kamyshnyi 2021) and to search for new targets for their therapy (Nosulenko et al. 2014). In this case, the analysis of transcriptome (Degen et al. 2020; Putilin et al. 2020; Bilous et al. 2021; Kamyshna et al. 2021c) and single nucleotide polymorphism (Kamyshna et al. 2021b) have been considered to be effective tools for identifying the hereditary predisposition to some conditions.

The aim of the present work was to define the risk factors that can affect the presence of a cognitive impairment and analyze the associations of the BDNF gene polymorphism (rs6265), the VDR gene polymorphism (rs2228570), and the N-methyl-D-aspartate (NMDA) receptor gene polymorphism (rs4880213) with the cognitive impairment in patients with autoimmune thyroiditis and hypothyroidism in the Western Ukraine population and predict the development of cognitive disorders in these patients.

Materials and methods

Our research was conducted in Bukovinian State Medical University, Chernivtsi Regional Endocrinology Center, and I. Horbachevsky Ternopil National Medical University, Ukraine. The study included 153 patients with different types of thyroid pathology. All of them were women. The subjects were divided into three groups: group 1 (n=16) comprised of patients with postoperative hypothyroidism (PO); group 2 (n=65) included patients with hypothyroidism (H) caused by autoimmune thyroiditis (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 randomly selected as a control group without adjusting for age or gender.

Ethical approval. The study fully ensured the standards described in the Helsinki Declaration of Human Rights 1975 (amended in 2008). The participants completed and signed a written consent before being voluntarily enrolled in the research. The Ethics Committee of the HSEEU "Bukovinian State Medical University", I. Horbachevsky Ternopil National Medical University and Chernivtsi Regional Endocrinology Center, Ukraine, have approved this study under an approval ID: 11-07.11.2017.

Assessment of thyroid gland function. In the hypothyroidism diagnosis, we were guided by the requirements of the American Association of Clinical Endocrinologists 2012. The corresponding clinical features were considered when verifying AIT, namely the results of a sonogram of the thyroid gland (reduced echogenicity) and circulating antibodies to thyroid antigens, anti-TPO, and anti-TG were detected (Garber et al. 2012).

Blood samples from patients and controls were taken in the morning (8:00 to 10:00 a.m.) after an overnight fasting. The 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 (TSH, normal range 0.3–4.0 mIU/mL), anti-thyroid peroxidase (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) in each individual who participated in the study.

The study exclusion criteria were the following: less than 18 years of age, malignancy, inflammation resulting from rheumatic diseases or acute/chronic infection, diabetes mellitus, vascular and chronic diseases of liver and kidneys, and pregnancy. Individuals administering drugs that could influence thyroid function were also ruled out from the study.

Assessment of cognitive function, depression, and anxiety. We detected a decline in the cognitive function using the Mini-Mental State Examination (MMSE), which has been the most used screening instrument throughout decades (Batty et al. 2013; Bos et al. 2015). We identified the severity of

depression and anxiety levels using the Hamilton Depression Rating Scale (HAM-D; Hamilton 1967) and Hamilton Rating Scale for anxiety (HAM-A; Hamilton 1959). Due to the HAM-D, scores of 0–7, 8–13, 14–18, 19–23, and 23–53 were considered as normal, mild, medium, severe, and very severe, respectively (Blacker 2007). The Hamilton Rating Scale for Anxiety (HAM-A; Hamilton 1959) is one of the most widely used values for assessing the anxiety severity in the research settings. Due to the HAM-A, each item was scored on a scale of 0 (not present) to 4 (severe) with a total score range of 0–56, where <17 indicates mild severity, 18–24 mild to moderate severity, and 25–30 moderate to severe.

Enzyme-linked immunosorbent assays (ELISA). BDNF levels in the serum of patients and healthy individuals were quantified by a highly sensitive Human BDNF ELISA Kit (Elabscience®, United States, Catalog No: E-EL-H0010) using E.I.A. Reader Sirio S (Seac, Italy).

Glutamate Receptor (Ionotropic, N-Methyl-D-Aspartate 2B) GRIN2B levels in the serum of patients and healthy subjects were assessed by to Human highly sensitive GRIN2B ELISA Kit (Elabscience®, United States, Catalog No E-EL-H1631).

For determining 25-OH Vitamin D levels in the serum of patients and healthy individuals, 25-OH Vitamin D Total (Vit D-Direct) Test System ELISA Kit (Monobind Inc.®, United States, Product Code: 9425-300) using E.I.A. Reader Sirio S (Seac, Italy) was applied.

Genotyping of the VDR (rs2228570), BDNF (rs6265), and NMDA receptor (rs4880213) gene polymorphism.

DNA isolation. The venous blood was collected in a sterile Vacutainer stabilized with K2EDTA. The total DNA was isolated from the peripheral blood by PREP-RAPID-GENETICS DNA Extraction Kit (DNA-TECHNOLOGY, Russian Federation, Catalog No P-021/4) according to the manufacturer's instructions.

DNA amplification and genotyping. The samples were genotyped by TaqMan probes and TaqMan Genotyping Master Mix (4371355) on CFX96™Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., USA). Polymerase chain reaction (PCR) for TaqMan genotyping was conducted due 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 intensified in a 10 μL reaction mix comprising genomic DNA, forward and reverse primers, fluorescent probes, and TaqMan Genotyping Master Mix. Genotyping of the samples conducted on the CFX-Manager ™ software using allelic discrimination assays based on the magnitude of relative fluorescence units.

Statistical analysis. We used the Student's t-test, ANOVA, Pearson's χ^2 test, ROC-analysis, odds ratio test, relative odds ratio test, and equality 0 correlation test to determine the difference between groups. The odds ratio and 95% confidence interval (CI) were computed by binary logistic regression where p values <0.05 were regarded as a statistically significant difference between the two groups (Statsoft Statistica v.12.0).

Results

The demographic, clinical, and biochemical characteristics of the participants are displayed in Table 1. In our study, we evaluated cognitive impairment in the examined patients based on the results of the MMSE test (Table 2). According to our study, in 50% of patients with PO no cognitive impairment was detected according to the MMSE test, whereas 37.5% of patients in this group had moderate pre-dementia cognitive impairment and 12.5% mild dementia. In 49.2% of patients with AIT with hypothyroidism, cognitive impairment was not detected, while 30.8% of patients had moderate pre-dementia cognitive impairment, and 20% of patients in this group mild dementia. Moderate and severe dementia was not detected in the examined patients. In the group of AIT patients, only 16.7% showed moderate pre-dementia cognitive impairment. In the rest of the patients of this group, no pathology was detected according to the MMSE test.

Analysis of the cognitive impairment presence was performed conditioning on the polymorphism of the BDNF gene (rs6265). According to the data obtained, when comparing the presence of cognitive impairment according to the MMSE test (Table 3), we revealed statistically reliable differences depending on the polymorphism of the BDNF gene (rs6265) ($p<0.001$). Thus, probable changes were observed among carriers of CT and TT genotypes with carriers of CC genotype ($p<0.001$). Due to the presence of cognitive impairment according to the MMSE test, depending on the polymorphism of the VDR gene (rs2228570) and the polymorphism of the NMDA gene

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

Variables	Control group (n=25)	Patient groups		
		PO (n=16)	AIT with H (n=65)	AIT and elevated anti-Tg and anti-TPO antibodies (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±3.14 (p<0.001)	21.48±2.83 (p<0.001)
BDNF (pg/ml)	1037.80±361.83	310.10±112.84 (p<0.001)	329.34±312.57 (p<0.001)	1031.34±385.00 (p=0.95)

Abbreviations: AIT – autoimmune thyroiditis; anti-Tg – anti-thyroglobulin antibody; anti-TPO – anti-thyroid peroxidase antibody; fT4 – free thyroxine; H – hypothyroidism; PO – postoperative hypothyroidism; TSH – thyroid stimulating hormone. Data are presented as a mean ± standard deviation.

Table 2
Analysis of cognitive impairment on the MMSE test conditioning on groups of subjects.

Variable	Categories	Groups of subjects				p-value
		Control	PO	AIT with H	AIT	
Cognitive impairment on the MMSE test	No cognitive impairment	25 (100.0)	8 (50.0)	32 (49.2)	60 (83.3)	p<0.001* $p_{Control - PO}$ =0.002 $p_{Control - AIT with H}$ <0.001 $p_{PO - AIT}$ =0.003 $p_{AIT with H - AIT}$ <0.001
	Moderate (pre-dementia) cognitive impairment	0	6 (37.5)	20 (30.8)	12 (16.7)	
	Mild dementia	0	2 (12.5)	13 (20.0)	0 (0.0)	

Abbreviations: AIT – autoimmune thyroiditis; H – hypothyroidism; MMSE – Mini-Mental State Examination; PO – postoperative hypothyroidism. *p<0.05 – differences are statistically significant.

Table 3
Analysis of the presence of cognitive impairment conditioning on the polymorphism of the BDNF gene (rs6265), VDR gene (rs2228570), and NMDA gene (rs4880213).

Variables	Categories	Presence of cognitive impairment		p-value
		No cognitive impairment	Presence of cognitive impairment	
BDNF gene polymorphism (rs6265)	CC	105 (86.8)	16 (13.2)	p<0.001* $p_{CC - CT}$<0.001 $p_{CC - TT}$<0.001
	CT	17 (37.8)	28 (62.2)	
	TT	3 (25.0)	9 (75.0)	
VDR gene polymorphism (rs2228570)	AA	49 (72.1)	19 (27.9)	0.898
	AG	50 (68.5)	23 (31.5)	
	GG	26 (70.3)	11 (29.7)	
NMDA gene polymorphism (rs4880213)	CC	36 (66.7)	18 (33.3)	0.555
	CT	63 (74.1)	22 (25.9)	
	TT	26 (66.7)	13 (33.3)	

Abbreviations: BDNF – brain-derived neurotrophic factor; NMDA – N-methyl-D-aspartate; VDR – vitamin D receptor. *p<0.05 – differences are statistically significant.

(rs4880213), statistically not significant differences were found (p=0.898, p=0.555), respectively.

We conducted a cognitive impairment analysis on the MMSE scale conditioning on the polymorphism of the BDNF gene (rs6265). According to the data obtained, when comparing cognitive impairment on the MMSE scale (Table 4), statistically reliable differences were detected depending on BDNF gene polymorphism (rs6265) (p<0.001). Thus, among CC carriers of the genotype only 9.1% of the subjects showed moderate (pre-dementia) cognitive impairment, while in carriers of CT and TT genotypes, such changes were detected in 51.1% and 33.3%, respectively. Mild dementia was observed in 41.7% of carriers of the TT genotype, 11.1% of carriers of the CT genotype, and only 4.1% of carriers of the CC genotype (p=0.621, p=0.802) respectively.

Correlation analysis of the association between hormones levels and MMSE was performed (Table 5). Analysis of the correlation between cognitive impairment according to MMSE test and the levels of TSH, fT4, anti-TG, and anti-TPO antibodies, 25-OH Vitamin D, and BDNF levels showed a strong direct correlation between MMSE test and BDNF levels (p<0.001) and a direct moderate correlation between cognitive impairment and fT4 (p<0.001), and weak direct correlation between 25-OH Vitamin D levels (p<0.001). Furthermore, we revealed a moderate inverse correlation between the MMSE test and TSH in the blood (p<0.001) and GRIN2B (p=0.003). At the same time, we detected a weak inverse relationship between depression and anti-TPO (p<0.001) and anti-TG (p=0.035).

The observed dependence of the "MMSE" index on the "BDNF" index is described by the paired linear regression equation: $Y_{MMSE} = 0.003 \times X_{BDNF} + 24.278$. With an increase in BDNF by 1, an increase in MMSE by 0.003 should be expected. Following the coefficient of determination R^2, the obtained model considers 41.6% of the factors influencing the value of the "MMSE" index (Figure 1).

The dependence of the "MMSE" index on the "GRIN2B" index is described by the paired linear regression equation: $Y_{MMSE} = -0.227 \times X_{GRIN2B} + 28.18$. With a decrease in the "GRIN2B" index by 1, an increase in the "MMSE" index by -0.227 should be expected. By the coefficient of determination R^2, the resulting model considers 7.2% of the factors influencing the value of the "MMSE" index (Figure 2). The assessment of the dependence of the "MMSE" index on quantitative factors was performed by the multiple linear regression method (Table 6). The observed dependence of the MMSE index on the BDNF index, TSH index, the anti-TG index is described by the linear regression equation: $Y_{MMSE} = 28.507 + 0.002 \times X_{BDNF} - 0.269 \times X_{TSH} - 0.076 \times X_{anti\text{-}TG}$.

With an increase in the "BDNF" index by 1, an increase in the "MMSE" index by 0.002 should be expected, with an increase in the "TSH" index by 1, a decrease in the "MMSE" index by 0.269 should be expected, with an increase in the " anti-TG" index by 1, a decrease should be expected the "MMSE" index by 0.076. The resulting regression model is characterized by a correlation coefficient of rxy=0.7, which corresponds to a high correlation on the Chaddock

Table 4
Analysis of the cognitive impairment on the MMSE scale on the polymorphism of the BDNF gene (rs6265), VDR gene (rs2228570), and NMDA gene (rs4880213).

Variables	Categories	Cognitive impairment on the MMSE scale			p-value
		No cognitive impairment	Moderate (pre-dementia) cognitive impairment	Mild dementia	
BDNF gene polymorphism (rs6265)	CC	105 (86.8)	11 (9.1)	5 (4.1)	p<0.001* p_{CC-CT}<0.001 p_{CC-TT}<0.001 p_{CT-TT}=0.047
	CT	17 (37.8)	23 (51.1)	5 (11.1)	
	TT	3 (25.0)	4 (33.3)	5 (41.7)	
VDR gene polymorphism (rs2228570)	AA	49 (72.1)	12 (17.6)	7 (10.3)	0.621
	AG	50 (68.5)	19 (26.0)	4 (5.5)	
	GG	26 (70.3)	7 (18.9)	4 (10.8)	
NMDA gene polymorphism (rs4880213)	CC	36 (66.7)	12 (22.2)	6 (11.1)	0.802
	CT	63 (74.1)	16 (18.8)	6 (7.1)	
	TT	26 (66.7)	10 (25.6)	3 (7.7)	

Abbreviations: BDNF – brain-derived neurotrophic factor; MMSE – Mini-Mental State Examination; NMDA – N-methyl-D-aspartate; VDR – vitamin D receptor. *p<0.05 – differences are statistically significant.

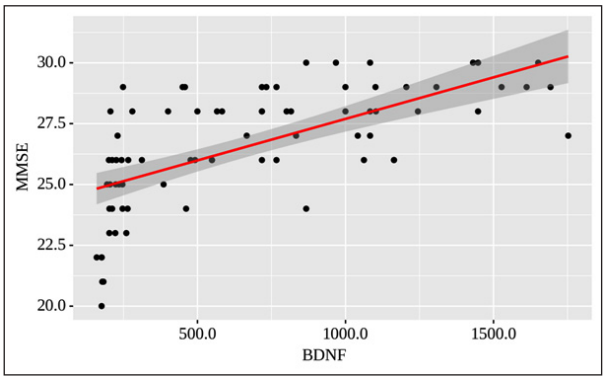


Figure 1. Regression line characterizing the dependence of Mini-Mental State Examination (MMSE) on brain-derived neurotrophic factor (BDNF).

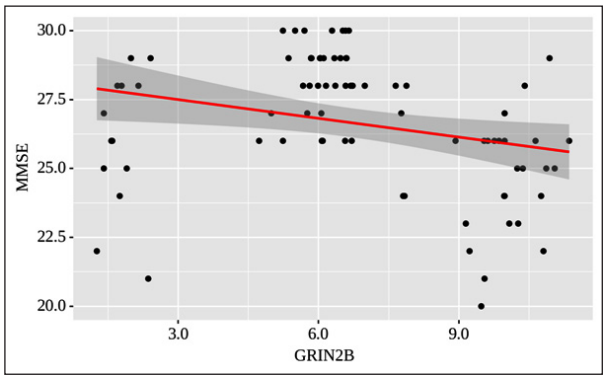


Figure 2. Regression line characterizing the dependence of Mini-Mental State Examination (MMSE) test on Glutamate Receptor (Ionotropic, N-Methyl-D-Aspartate 2B, GRIN2B).

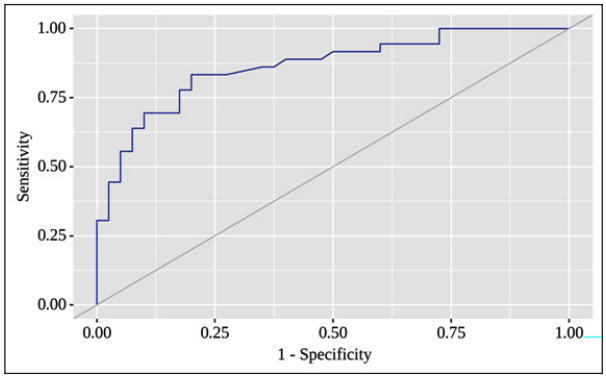


Figure 3. ROC-curve describes the dependence of the probable Presence of cognitive impairment on brain-derived neurotrophic factor (BDNF).

scale. The model was statistically reliable ($p<0.001$). Based on the value of the coefficient of determination R^2, the model considers 55.2% of the factors that determine the change in the "MMSE" index.

Table 5
Results of the correlation analysis of the association between the levels of hormones and cognitive impairment due to the MMSE test.

Variables	Correlation characteristics		
	p-value	The strength of the association was assessed using the Chaddock scale	p-value
BDNF – MMSE	0.714	Strong	<0.001*
GRIN2B – MMSE	–0.332	Moderate	0.003*
TSH – MMSE	–0.455	Moderate	<0.001*
fT4 – MMSE	0.428	Moderate	<0.001*
anti-TPO – MMSE	–0.296	Weak	<0.001*
anti-TG – MMSE	–0.166	Weak	0.035*
Vitamin D – MMSE	0.275	Weak	<0.001*

Abbreviations: anti-TG – anti-thyroglobulin antibody; anti-TPO – anti-thyroid peroxidase antibody; BDNF – brain-derived neurotrophic factor; fT4 – free thyroxine; GRIN2B – Glutamate Receptor, Ionotropic, N-Methyl-D-Aspartate 2B; MMSE – Mini-Mental State Examination; TSH – thyroid-stimulating hormone. *p<0.05 – differences are statistically significant.

Table 6
Analysis of the "MMSE" index depending on "BDNF", "TSH", "anti-TG".

	B	S.E.	T	p-value
intercept	28.507	1.038	27.459	<0.001*
BDNF	0.002	0.001	3.674	<0.001*
TSH	–0.269	0.101	–2.654	0.010*
anti-TG	–0.076	0.019	–3.920	<0.001*

Abbreviations: anti-TG – anti-thyroglobulin antibody; B – regression coefficient; BDNF – brain-derived neurotrophic factor; MMSE – Mini-Mental State Examination test; S.E. – standard error; T– Student's t-test; TSH – thyroid-stimulating hormone. *p<0.05 – differences are statistically significant.

We conducted a study of the prognostic value of plasma levels of a number of hormones as potential markers of cognitive impairment. The displayed curve was obtained during estimation of the dependence of detecting the presence of cognitive impairment conditioning on BDNF using ROC analysis. The following curve was gained when assessing the dependence of detecting the presence of cognitive impairment conditioning on BDNF using the ROC analysis (Figure 3).

The area under the ROC curve constituted 0.864±0.042 with 95% CI: 0.781–0.947. The resulting model was statistically reliable ($p<0.001$). The cut-off

value of BDNF, which is aligned with the highest Youden's J index, was 566.240. If BDNF was lower or equal to this value. Cognitive Impairment Detection was predicted. The sensitivity and specificity of the method constituted 83.3% and 80.0%, respectively.

For the development of cognitive impairment, prognostically significant indicators were also found to be TSH levels ($p<0.001$; sensitivity: 80.4%, specificity: 67.6%), fT4 ($p<0.001$; sensitivity: 86.3%, specificity: 59.5%) and vitamin D ($p=0.010$; sensitivity: 54.7%, specificity: 60.8%).

The construction of a predictive model of the probability of a certain outcome was carried out using a logistic regression method. The Nigelkirk's R^2 coefficient was a measure of certainty indicating that part of the variance can be explained using logistic regression.

To assess the diagnostic significance of quantitative signs in predicting a certain outcome, the analysis method of ROC curves was used. The separating value of the quantitative trait at the cut-off point was determined by the highest value of the Youden index.

A predictive model was developed to determine the likelihood of the presence of cognitive impairment depending on the VDR, the (rs4880213), the GRIN2B index, the rs6265, on "Hamilton depression rating scale" by the method of binary logistic regression. The observed dependence is described by the equation: P=1/(1+e–z)×100%; z=–7.162+0.365XAG–1.170XGG+0.310XCT+0.942XTT+1.843XGRIN2B+3.215XCT+3.079XTT+2.365X. Mild depressive disorder+1.575X and moderate depressive disorder +1.746X. The presence of anxiety on the Hamilton scale, where P is the probability of presence of cognitive impairment, XAG-VDR (0-AA, 1-AG), XGG-VDR (0-AA, 1-GG), XCT-(rs4880213) (0-CC, 1-CT), XTT-(rs4880213) (0-CC, 1-TT), XLIN level GRIN2)-GRIN2 level), XCT-rs6265 (0-CC, 1-CT), XTT-rs6265 (0-CC, 1-TT), X Mild depressive disorder - presence of depression on the Hamilton scale (0 – Norm, 1 – Mild depressive disorder), X depressive disorder of moderate severity – presence of depression on the Hamilton scale (0-Norm, 1- depressive disorder of moderate severity), X presence of anxiety - the presence of anxiety on the Hamilton scale.

The obtained regression model is statistically significant ($p<0.001$). Based on the value of the Nigelkirk coefficient of determination, the model explains 77.1% of the variance observed in the presence of cognitive impairment. When evaluating the complex model, based on the values of the regression coefficients, a direct relationship between the "Level GRIN2B" index and cognitive impairments was established (Table 7). Thus, the likelihood of cognitive impairment increased by 6.317 ($p=0.006$). When assessing the effect of the CT rs6265 genotype, an increase in the likelihood of cognitive impairment by 24.898-fold ($p=0.012$) was also showed. In addition, a direct relationship was also established between the influence of the TT rs6265 genotype and an increase in the likelihood of cognitive impairment by a factor of 21.734 ($p=0.024$) (Figure 4).

When assessing the dependence of the probability of the presence of cognitive impairments on the value of the logistic function P using the ROC analysis, the following curve was obtained (Figure 5). The area under the ROC curve was 0.956±0.025 with 95% CI: 0.907–1.000. The resulting model was statistically significant ($p<0.001$).

The threshold value of the logistic function P at the cut-off point, which corresponded to the highest value of the Youden's index, was 0.729. The appearance of cognitive impairments was predicted when the value of the logistic function P was higher or equal to this value. The sensitivity and specificity of the model were 83.3% and 95.0%, respectively.

Discussion

In the present study, a strong direct relationship between the GRIN2B levels and cognitive impairments was found after evaluating the complex model based on the values of the regression coefficient and an increase in the likelihood of cognitive impairment after assessing the effect of the CT rs6265 genotype. In addition, direct relationship between the influence of the TT rs6265 genotype and an increase in the likelihood of cognitive impairment was also established. The present data also indicate that the BDNF, TSH, fT4, and vitamin D levels may prognostically belong to the significant indicators of the cognitive impairment development.

Deteriorated cognitive function, memory impairment, and depressive symptoms have been observed in the adult patients brain with hypothyroidism (Lass et al. 2008). Patients who received T4 to overcome hypothyroidism were not always fully cured of symptoms that impact the central nervous system (CNS), such as the memory capacity (Wekking et al. 2005).

Our previous studies have shown that autoimmune thyroiditis (AIT) and hypothyroidism can affect the neuropeptides genes transcription and their receptors (Kamyshna et al. 2021c), the cell cycle

Table 7
Characteristics of the relationship of model predictors with the probability of detecting the presence of cognitive impairment.

Predictors	Unadjusted		Adjusted	
	COR (95% CI)	p-value	AOR (95% CI)	p-value
VDR: AG	1.667 (0.647–4.297)	0.290	1.440 0.200–10.381)	0.718
VDR: GG	0.689 (0.218–2.173)	0.525	0.310 (0.033–2.936)	0.308
(rs4880213): CT	0.596 (0.240–1.481)	0.266	1.363 (0.120–15.456)	0.802
(rs4880213): TT	1.619 (0.502–5.223)	0.420	2.565 (0.131–49.999)	0.534
GRIN2B	5.750 (2.790–11.858)	<0.001*	6.317 (1.696–23.524)	0.006*
(rs 6265): CT	3.082 (1.146–8.290)	0.026*	24.898 (2.050–302.475)	0.012*
(rs6265): TT	3.524 (0.856–14.498)	0.081	21.734 (1.499–315.135)	0.024*
Presence of depression on the Hamilton scale: Mild depressive disorder	0.818 (0.170–3.931)	0.802	10.645 (0.326–347.930)	0.184
Presence of depression on the Hamilton scale: Depressive disorder of moderate severity	18.666 (5.403–64.522)	<0.001*	4.830 (0.245–95.393)	0.301
The presence of anxiety on the Hamilton scale	15.596 (5.028–48.376)	<0.001*	5.734 (0.845–38.900)	0.074

Abbreviations: AOR – adjusted odds ratio; COR – crude odds ratio; GRIN2B – Glutamate Receptor, Ionotropic, N-Methyl-D-Aspartate 2B; VDR – vitamin D receptor. *p<0.05 – association of the outcome value with the predictor value is statistically significant.

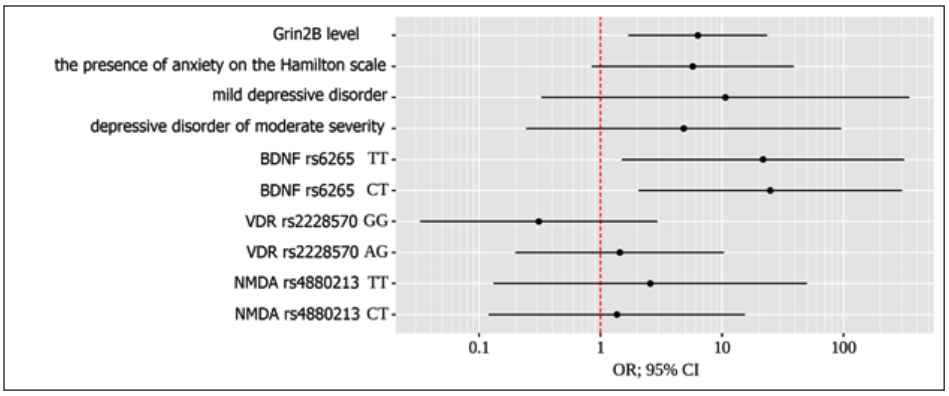


Figure 4. Estimation of the odds ratio with the corresponding 95% CI's for the predictors included in the model Presence of cognitive impairment.

regulation (Bilous et al. 2020a), and the transmission of nerve impulses (Bilous et al. 2020b). Such changes in gene expression can contribute to the development of neurological complications related to the thyroid pathology (Kamyshna et al. 2021a,d). Due to the high incidence of hypothyroidism in the human population, there is a rising necessity to understand the cellular and molecular mechanisms altered by this disorder.

In humans, an investigation of normal subjects has shown that carriage of the Met66 allele of the BDNF Val66Met polymorphism is connected with the poorer episodic memory, atypical patterns of hippocampal activation (as estimated by functional magnetic resonance imaging), and lesser n-acetyl aspartate levels in the hippocampus (analyzed by functional magnetic resonance imaging spectroscopy) (Egan et al. 2003) assuming that this

polymorphism can be related to specific cognitive functions.

Recently, a relationship between BDNF Val66Met polymorphism and hippocampal activity during episodic memory processing by blood oxygenation level-dependent functional magnetic resonance imaging and a declarative memory task in a healthy population has been reported (Hariri et al. 2003). The outcomes have shown that the Met66 allele carriers revealed a relatively reduced hippocampal involvement than the Val66 homozygotes during encoding and retrieval processes. The interaction between the BDNF Val66Met polymorphism and the hippocampal response during encoding constituted one-fourth of the total variation in the recognition memory performance.

Numerous (Harris et al. 2006; Schofield et al. 2009), but not all (Tsai et al. 2008; Houlihan et al. 2009) studies have revealed reliable evidence of the connection between BDNF Val66Met polymorphism and cognition. In our study, statistically reliable differences were detected depending on the BDNF gene (rs6265) ($p<0.001$) polymorphism. Thus, among CC carriers of the genotype only 9.1% of the subjects showed moderate (pre-dementia) cognitive impairment, while in carriers of CT and TT genotypes, such changes were detected in 51.1% and 33.3%, respectively. Mild dementia was observed in 41.7% of carriers of the TT genotype, 11.1% the CT genotype and only 4.1% the SS genotype ($p=0.621$, $p=0.802$, respectively).

Vitamin D deficiency was connected with AITD and HT along with the progress of thyrocyte damage in HT and a negative correlation between the vitamin D levels and TSH after considering age, sex, body mass index, and sampling season has been noticed (Kim 2016). In our study, analysis of the correlation between cognitive impairment, according to the MMSE test and the levels of 25-OH Vitamin D and BDNF levels, showed a weak direct correlation between 25-OH Vitamin D levels ($p<0.001$) and a strong direct correlation between the MMSE test and BDNF levels ($p<0.001$).

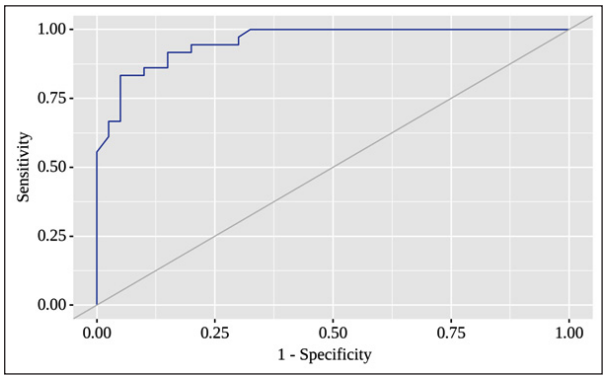


Figure 5. ROC-curve displaying the dependence of the presence of cognitive impairment feasibility on the logistic function p-value.

The specific role of vitamin D in the pathophysiology of cognitive impairment in patients with HT has not yet been clarified. A probable elucidation is vitamin D effect on the inflammatory cytokines. Earlier studies have described an increased cytokine production, which includes interleukin-1β (IL-1β), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-alpha (TNF-α), in patients with HT (Mikos et al. 2014; Sun et al. 2016). Vitamin D has been implicated in modulating the cytokines secretion, including TNF-α, IL-6, IL-1β, and MCP-1 (Farhangi et al. 2017). Various studies have indicated that they are in a favor of the notion that inflammation characterized by elevated cytokine levels may play a crucial role in the cognitive impairment pathogenesis (Takeda et al. 2014). These outcomes presuppose that vitamin D might play a vital role in the cognitive impairment in the HT patients.

A meta-analysis of vitamin D receptor gene polymorphisms and AITD has shown a significant correlation between specific vitamin D receptor gene polymorphisms (such as BsmI and TaqI) and autoimmune thyroid diseases (Feng et al. 2013). In our study, due to the presence of cognitive impairment according to the MMSE test depending on the polymorphism of the VDR gene (rs2228570), statistically not significant differences were found.

N-methyl-D-aspartate receptors (NMDARs) are a family of ionotropic glutamate receptors that mediate a slow, $Ca2^{+}$ permeable component of excitatory synaptic transmission in the central nervous system (CNS). NMDARs are a tetrameric assembly of two glycine-binding GluN1 subunits and two glutamate-binding GluN2 subunits (Saito et al. 2020).

In the light of an important role of GRIN2B in controlling NMDAR function, the time course of synaptic currents and plasticity, mutation-related disruption in the GluN2B subunit expression or function might give a rise to several neurodevelopmental phenotypes that reflect alterations in circuit formation, neuronal connectivity, synaptic plasticity, and excitatory transmission. Some variants in the NMDAR gene family with the GRIN2B gene encoding the GluN2B subunit being implicated in many cases of neurodevelopmental disorders, which are psychiatric conditions originating in childhood

and include language, motor and learning disorders, autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), developmental delay, epilepsy, and schizophrenia have been found (Endele et al. 2010; Hu at al. 2017). The GRIN2B gene has been shown to play a crucial role in the normal neuronal development and is important for learning and memory (Reutlinger et al. 2010).

It has also been noticed at the molecular level that hypothyroidism contributes to the altered expression of mRNA encoding subunits comprising the N-methyl-D-aspartic acid receptor (NMDAR) in adult rats (Lee et al. 2003). We did not reveal significant differences between the polymorphism of the NMDA gene (rs4880213) and cognitive impairment according to the MMSE test. At the same time, we revealed a moderate inverse correlation between the cognitive impairment on the MMSE scale and the level of GRIN2B (p=0.003).

Conclusion

When evaluating the complex model based on the values of the regression coefficients, a strong direct relationship was established between the "GRIN2B Level" and cognitive impairments. When assessing the effect of the CT rs6265 genotype, an increase in the likelihood of cognitive impairment by 24.898-fold was seen. In addition, direct relationship was also established between the influence of the TT rs6265 genotype and an increase in the likelihood of cognitive impairment by a factor of 21.734. The levels of BDNF ($p<0.001$), TSH ($p<0.001$), fT4 ($p<0.001$), and vitamin D ($p=0.01$) prognostically belong to the significant indicators of the cognitive impairment development.

***Conflict of interest**: The authors declare no conflict of interest.*

References

Annweiler C, Dursun E, Féron F, Gezen-Ak D, Kalueff AV, Littlejohns T, Llewellyn DJ, Millet P, Scott T, Tucker KL, Yilmazer S, Beauchet O. Vitamin D and cognition in older adults: updated international recommendations. J Intern Med 277, 45–57, 2015.

Batty GD, Li Q, Huxley R, Zoungas S, Taylor BA, Neal B, de Galan B, Woodward M, Harrap SB, Colagiuri S, Patel A, Chalmers J; VANCE Collaborative group. Oral disease in relation to future risk of dementia and cognitive decline: prospective cohort study based on the action in diabetes and vascular disease: preterax and diamicron modified-release controlled evaluation (ADVANCE) trial. Eur Psychiatry 28, 49–52, 2013.

Bilous I, Pavlovych L, Krynytska I, Marushchak M, Kamyshnyi A. Apoptosis and cell cycle pathway-focused genes expression analysis in patients with different forms of thyroid pathology. J Med Sci 8, 784–792, 2020a.

Bilous II, Korda MM, Krynytska IY, Kamyshnyi AM. Nerve impulse transmission pathway-focused genes expression analysis in patients with primary hypothyroidism and autoimmune thyroiditis. Endocr Regul 54, 109–118, 2020b.

Bilous II, Pavlovych L, Kamyshnyi A. Primary hypothyroidism and autoimmune thyroiditis alter the transcriptional activity of genes regulating neurogenesis in the blood of patients. Endocr Regul 55, 101–111, 2021.

Blacker D. Rating scales in psychiatry. In: Sadock BJ, Sadock VA (Eds.). Comprehensive Textbook of Psychiatry (8th). Lippincott Williams and Wilkins, Philadelphia, pp. 929–943, 2007.

Bos D, Vernooij MW, de Bruijn RF, Koudstaal PJ, Hofman A, Franco OH, van der Lugt A, Ikram MA. Atherosclerotic calcification is related to a higher risk of dementia and cognitive decline. Alzheimers Dement 11, 639–647, 2015.

Degen AS, Krynytska IY, Kamyshnyi AM. Changes in the transcriptional activity of the entero-insular axis genes in streptozotocin-induced diabetes and after the administration of TNF-α non-selective blockers. Endocr Regul 54, 160–171, 2020.

Djurovic M, Pereira AM, Smit JWA, Vasovic O, Damjanovic S, Jemuovic Z, Pavlovic D, Miljic D, Pekic S, Stojanovic M, Asanin M, Krljanac G, Petakov M. Cognitive functioning and quality of life in patients with Hashimoto thyroiditis on long-term levothyroxine replacement. Endocrine 62, 136–143, 2018.

Dzhuryak V, Sydorchuk L, Sydorchuk A, Kamyshnyi O, Kshanovska A, Levytska S, Knut R, Sheremet M, Ivashchuk S, Petrynych O, Kazantseva T, Nikyfor L, Melnychuk L, Sokolenko A, Yarynych Y, Semianiv M, Repchuk Y, Voroniuk K, Sydorchuk R, Sokolenko L, Iftoda O, Kushnir O. The cytochrome 11B2 aldosterone synthase gene CYP11B2 (RS1799998) polymorphism associates with chronic kidney disease in hypertensive patients. Biointerface Res Appl Chem 10, 5406–5411, 2020.

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112, 257–269, 2003.

Endele S, Rosenberger G, Geider K, Popp B, Tamer C, Stefanova I, Milh M, Kortüm F, Fritsch A, Pientka FK, Hellenbroich Y, Kalscheuer VM, Kohlhase J, Moog U, Rappold G, Rauch A, Ropers HH, von Spiczak S, Tönnies H, Villeneuve N, Villard L, Zabel B, Zenker M, Laube B, Reis A, Wieczorek D, Van Maldergem L, Kutsche K. Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. Nat Genet 42, 1021–1026, 2010.

Farhangi MA, Mesgari-Abbasi M, Hajiluian G, Nameni G, Shahabi P. Adipose tissue inflammation and oxidative stress: the ameliorative effects of vitamin D. Inflammation 40, 1688–1697, 2017.

Feng M, Li H, Chen SF, Li WF, Zhang FB. Polymorphisms in the vitamin D receptor gene and risk of autoimmune thyroid diseases: a meta-analysis. Endocrine 43, 318–326, 2013.

Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, Pessah-Pollack R, Singer PA, Woeber KA, American Association of Clinical Endocrinologists and American Thyroid Association Taskforce on Hypothyroidism in Adults. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Endocr Pract 18, 988–1028, 2012.

Hamilton M. The assessment of anxiety states by rating. Br J Med Psychol 32, 50–55, 1959.

Hamilton M. Development of a rating scale for primary depressive illness. Br J Soc Clin Psychol 6, 278–296, 1967.

Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF, Weinberger DR. Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. J Neurosci 23, 6690–6694, 2003.

Harris SE, Fox H, Wright AF, Hayward C, Starr JM, Whalley LJ, Deary IJ. The brain-derived neurotrophic factor Val-66Met polymorphism is associated with age-related change in reasoning skills. Mol Psychiatry 11, 505–513, 2006.

Houlihan LM, Harris SE, Luciano M, Gow AJ, Starr JM, Visscher PM, Deary IJ. Replication study of candidate genes for cognitive abilities: the Lothian Birth Cohort 1936. Genes Brain Behav 8, 238–247, 2009.

Hu C, Chen W, Myers SJ, Yuan H, Traynelis SF. Human GRIN2B variants in neurodevelopmental disorders. J Pharmacol Sci 132, 115–121, 2016.

Hung PL, Hsu MH, Yu HR, Wu KLH, Wang FS. Thyroxin protects white matter from hypoxic-ischemic insult in the immature Sprague-Dawley rat brain by regulating periventricular white matter and cortex BDNF and CREB pathways. Int J Mol Sci 19, 2573, 2018.

Kamyshna I, Kamyshnyi A. Transcriptional activity of neurotrophins genes and their receptors in the peripheral blood in patients with thyroid diseases in Bukovinian population of Ukraine. J Med Sci (Maced open access) 9, 208–216, 2021.

Kamyshna II, Pavlovych L, Kamyshnyi A. 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. J Med Sci (Maced open access) 9, 659–664, 2021a.

Kamyshna II, Pavlovych L, Malyk I, Kamyshnyi A. 25-OH Vitamin D blood serum linkage with VDR gene polymorphism (rs2228570) in thyroid pathology patients in the West-Ukrainian population. J Med Life 14, 549–556, 2021b.

Kamyshna II, Pavlovych LB, Maslyanko VA, Kamyshnyi AM. Analysis of the transcriptional activity of genes of neuropeptides and their receptors in the blood of patients with thyroid pathology. J Med Life 14, 243–249, 2021c.

Kamyshna II, Pavlovych LB, Sydorchuk LP, Malyk IV, Kamyshnyi AM. BDNF blood serum linkage with BDNF gene polymorphism (rs6265) in thyroid pathology patients in the West-Ukrainian population. Endocr Regul 55, 193–203, 2021d.

Kim D. Low vitamin D status is associated with hypothyroid Hashimoto's thyroiditis. Hormones (Athens) 15, 385–393, 2016.

Lass P, Slawek J, Derejko M, Rubello D. Neurological and psychiatric disorders in thyroid dysfunctions. The role of nuclear medicine: SPECT and PET imaging. Minerva Endocrinol 33, 75–84, 2008.

Lee PR, Brady D, Koenig JI. Thyroid hormone regulation of N-methyl-D-aspartic acid receptor subunit mRNA expression in adult brain. J Neuroendocrinol 15, 87–92, 2003.

LeMoult J, Gotlib IH. Depression: A cognitive perspective. Clin Psychol Rev 69, 51–56, 2019.

Leyhe T, Mussig K. Cognitive and affective dysfunctions in autoimmune thyroiditis. Brain Behav Immun 41, 261–266, 2014.

Mikos H, Mikos M, Rabska-Pietrzak B, Niedziela M. The clinical role of serum concentrations of selected cytokines: IL-1β, TNF-α and IL-6 in diagnosis of autoimmune thyroid disease (AITD) in children. Autoimmunity 47, 466–472, 2014.

Nosulenko IS, Voskoboynik OY, Berest GG, Safronyuk SL, Kovalenko SI, Kamyshnyi OM, Polishchuk NM, Sinyak RS, Katsev AV. Synthesis and Antimicrobial Activity of 6-Thioxo-6,7-dihydro-2H-[1,2,4]triazino[2,3-c]-quinazolin-2-one derivatives. Sci Pharm 82, 483–500, 2014.

Putilin DA, Evchenko SY, Fedoniuk LY, Tokarskyy OS, Kamyshny OM, Migenko LM, Andreychyn SM, Hanberher II, Bezruk TO. The influence of metformin to the transcriptional activity of the mTOR and FOX3 genes in parapancreatic adipose tissue of streptozotocin-induced diabetic rats. J Med Life 13, 50–55, 2020.

Reutlinger C, Helbig I, Gawelczyk B, Subero JI, Tönnies H, Muhle H, Finsterwalder K, Vermeer S, Pfundt R, Sperner J, Stefanova I, Gillessen-Kaesbach G, von Spiczak S, van Baalen A, Boor R, Siebert R, Stephani U, Caliebe A. Deletions in 16p13 including GRIN2A in patients with intellectual disability, various dysmorphic features, and seizure disorders of the rolandic region. Epilepsia 51, 1870–1873, 2010.

Sable HJ, MacDonnchadh JJ, Lee HW, Butawan M, Simpson RN, Krueger KM, Bloomer RJ. Working memory and hippocampal expression of BDNF, ARC, and P-STAT3 in rats: effects of diet and exercise. Nutr Neurosci 16, 1–14. 2021.

Saito T, Chiba Y, Abe K, Hattori S, Katsuse O, Takahashi Y, Suda A. An investigative study of antibodies to NMDA-type glutamate receptor subunits in serum and cerebrospinal fluid in psychiatric patients with anti-thyroid antibodies. Helion 6, e05677, 2020.

Schofield PR, Williams LM, Paul RH, Gatt JM, Brown K, Luty A, Cooper N, Grieve S, Dobson-Stone C, Morris C, Kuan SA, Gordon E. Disturbances in selective information processing associated with the BDNF Val66Met polymorphism: evidence from cognition, the P300 and fronto-hippocampal systems. Biol Psychol 80, 176–188, 2009.

Sun L, Zhang X, Dai F, Shen J, Ren C, Zuo C, Zhang Q. Elevated interleukin-1β in peripheral blood mononuclear cells contributes to the pathogenesis of autoimmune thyroid diseases, especially of Hashimoto thyroiditis. Endocr Res 41, 185–192, 2016.

Takeda S, Sato N, Morishita R. Systemic inflammation, blood-brain barrier vulnerability and cognitive/non-cognitive symptoms in Alzheimer disease: relevance to pathogenesis and therapy. Front Aging Neurosci 6, 171, 2014.

Tsai SJ, Gau YT, Liu ME, Hsieh CH, Liou YJ, Hong CJ. Association study of brain-derived neurotrophic factor and apolipoprotein E polymorphisms and cognitive function in aged males without dementia. Neurosci Lett 12, 433, 158–162, 2008.

Wekking EM, Appelhof BC, Fliers E, Schene AH, Huyser J, Tijssen JG, Wiersinga WM. Cognitive functioning and well-being in euthyroid patients on thyroxine replacement therapy for primary hypothyroidism. Eur J Endocrinol 153, 747–753, 2005.

Xu J, Zhu XY, Sun H, Xu XQ, Xu SA, Suo Y, Cao LJ, Zhou Q, Yu HJ, Cao WZ. Low vitamin D levels are associated with cognitive impairment in patients with Hashimoto thyroiditis. BMC Endocr Disord 18, 87, 2018.