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COMBINED EFFECT OF GENE-GENE INTERACTION ON THE AMONG THE FUNCTION OF THYROID GLAND AND THE DEGREE OF ITS ENLARGEMENT IN PATIENTS OF AUTOIMMUNE THYROIDITIS AND THYROID ADENOMA IN THE INHABITANTS OF NORTHERN BUKOVINA

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Abstract

Was a comprehensive analysis of combined influence of the gene polymorphisms associated with various links in the regulation of apoptosis (BCL-2, CTLA-4 and APO-1 / Fas) on the development of nodular goiter on the background of autoimmune thyroiditis and thyroid adenoma in the surveyed population with regard to changes in the functional activity of the thyroid gland and the degree of the thyroid gland enlargement has been performed. For this purpose, we used the method of Multifactor Dimensions Reduction 3.0.2 – MDR by calculating the prediction potentials.

Established that graphic models of gene-gene interaction with the highest cross-validation consistency created by Multifactor Dimensions Reduction 3.0.2 – MDR method, showed complex "synergistic or independent" impact of polymorphic loci of the CTLA-4 (+49G / A), Fas (-1377G / A) and Bcl-2 (63291411A> G) genes on the onset of the change of thyroid function and degree of hyperplasia in the population of residents of Northern Bukovina.

Key words: nodular goiter, autoimmune thyroiditis, thyroid adenoma, APO-1 / FAS, CTLA-4 and BCL-2 genes interaction.

Abbreviations: NGAIT - nodular goiter with autoimmune thyroiditis, AIT- autoimmune thyroiditis, TG - thyroid gland, TA - thyroid adenoma.

Introduction

The etiology of Hashimoto's thyroiditis is considered to be multifactorial, involving the interplay of various environmental and genetic factors. Studies conducted on the genetic associations of Hashimoto's thyroiditis have shown that the human leukocyte antigen (HLA) region, which plays a major role in other autoimmune disorders, is associated with development of Hashimoto's thyroiditis [1-5].

Defects in genes that play an important role in thyroid physiology and thyroid hormone synthesis could predispose to the development of goiter, especially in case of borderline or overt iodine deficiency. Such defects could lead to dyshormonogenesis as an immediate response, thereby indirectly explaining the nodular transformation of the thyroid as late consequences of dyshormonogenesis, as a form of maladaptation [6, 7]. The genes that encode the proteins involved in thyroid hormone synthesis, such as the thyroglobulin-gene (TG-gene), the thyroid peroxidase-gene (TPO-gene), the sodium – iodide – symporter-gene (SLC5A5), the Pendred syndrome-gene (SLC26A4), the TSH receptor-gene (TSH-R-gene), the iodothyronine deiodinase (DEHAL 1) and the thyroid oxidase 2 gene³ (DUOX2) are convincing candidate genes in familial euthyroid goiter [6]. Originally, several mutations in these genes were identified in patients with congenital hypothyroidism [7]. However, in cases of less severe functional impairment, which can still be compensated, a contribution of variants of these genes in the etiology of nontoxic goiter is possible.

The genetic predisposition to the development of AIT is confirmed by its association with certain antigens of the HLA system, but it should be noted that the antigens of the HLA system are markers of a number of autoimmune diseases, so they cannot be considered as a specific "disease gene" [8-11]. One can only talk about the innate propensity to a certain type of autoimmune reactions. Many studies have been conducted to find associations between autoimmune CHD diseases and a multitude of immune response loci, including the HLA region on the chromosome 6p21, the CTLA4 region on the chromosome 2q33, and the PTPN22 region on the chromosome 1 p1 [12-16].

The next step of our study was the comprehensive analysis of combined influence of the gene polymorphisms associated with various links in the regulation of apoptosis (BCL-2, CTLA-4 and APO-1 / Fas) on the functional activity changes (normal function, subclinical and clinical hypothyroidism) and TG hyperplasia degrees (IB, II and III) in the surveyed population. For this purpose, we used the method of Multifactor Dimensions Reduction 3.0.2 – MDR by calculating the prediction potentials.

Material and methods

95 women with NGAIT were examined in 2013- 2016 in Chernivtsi regional clinical hospital. The age of patients ranged from 23 to 72 years. The diagnosis was made clinically, in laboratory (thyroid peroxidase antibodies (TPAB) - 60-250 U / ml thyroglobulin antibodies (TGAB) - 60-500 U / ml; thyroid-stimulating hormone (TSH) - 4.10 mU / L), using ultrasound and it was confirmed histologically after the surgery.

We singled out a group of 30 women who had been diagnosed with thyroid adenoma after the surgery by ultrasound, fine-needle aspiration biopsy (FNAB) and histological conclusion. The group was identified due to the fact that this pathology is one of the most common forms of nodular goiter. These indicators served as controls. The final confirmation of morphologically unchanged tissue was obtained after the histological conclusion.

The TG functional activity changes (normal function, subclinical and clinical hypothyroidism). The diagnosis is confirmed biochemically by a reduction in serum free T4 with increased serum thyroid-stimulating hormone (TSH; thyrotropin). Subclinical hypothyroidism is a biochemical diagnosis and describes an elevated serum TSH with normal free thyroid hormones. Hypothyroidism is treated by T4 replacement, the aims of treatment being relief of symptoms and restoration of serum TSH to the reference range.

TG hyperplasia degrees (I and II) were analyzed according to the World Health Organization (WHO) classification:

Grade 0: No goiter is palpable or visible.

I degree: palpable goiter, not visible when neck is held in normal position

II degree: a clearly swollen neck (also visible in normal position of the neck) that is consistent with a goiter on palpation.

25 practically healthy donors were examined as well. 10 ml of the peripheral blood drawn from the ulnar vein in the morning on an empty stomach served as the material for the study.

All the patients underwent a surgery. The range of surgical intervention – from hemithyroidectomy to thyroidectomy.

The DNA was isolated by means of a set of reagents Thermo Scientific Gene JET Genomic DNA Purification kit (# K0721, Thermo Fisher Scientific), according to the directions for use, with incubation with proteinase K overnight to complete cell lysis. The purified DNA was diluted in Elution Buffer and evaluated with a spectrophotometer Nanodrop2000C. Only samples with a concentration of not lower than 15 ng / ml and the values of the ratio of A (260/280) between 1.7 and 2.0 were used for genotyping. The obtained extracts were divided into aliquots, one of which was placed in a refrigerator at 4°C until its use, while others were frozen at -20°C.

To normalize the amount of DNA, all samples were brought to a concentration of 2 ng / µL using Nuclease-free water.

To genotype the selected point polymorphism, the TaqMan technology was used. Polymorphisms marked with the reference number SNP ID, according to the database dbSNP have been studied. To test each of the polymorphisms TaqMan® SN Genotyping Assays (40X) (4,351,379, Thermo Fisher Scientific) were used (table 1)

Table 1

The nucleotide sequence of the region including the analyzed polymorphism

Reference number SNP ID	Test number (Assay ID*)	Fragment of the region including the analyzed polymorphism
rs231775 (CTLA4)	C_2415786_20	GCACAAGGCTCAGCTGAACCTGGCT[A/G]CCAGGACCTGGCCCTGCACTCTCCT
rs17759659 (BCL2)	C_33628167_10	TCTTCTTACCAAAGATTCACAATAC[A/G]GTGTGATGGGAACGTGACCTAGTT
rs2234767 (FAS)	C_12123966_10	CAGAGTGTGTGCACAAGGCTGGCAC[A/G]CCAGGGTCTTCCTCATGGCACTAA

Note: according to the website www.thermofisher.com.

The volume of the reaction mixture was 5 µL and consisted of: 2.5 µL reagent Taq Man Genotyping Master Mix (20X) (4,371,355, Thermo Fisher Scientific), 0,25 µL of probe solution and 2.25 µL of DNA solution. Genotyping was performed with the instrument Quant Studio 6 (Applied Biosystems, Thermo Fisher Scientific), 384-well block.

Amplification was performed under the following conditions:

Activation	10 min	95°C	
Denaturation	15 sec	92°C	40*/60**
Annealing / elongation	1 min	60°C	cycles

Note: * - to amplify the polymorphisms associated with CTLA-4 and Fas genes;

** - to amplify the polymorphisms associated with Bcl-2 genes.

To collect and process the data the software *Quant Studio™ Real-Time PCR* (v.1.3) was used.

The main part of the statistical analysis was carried out using the software «Statistica 7.0» (SPSS). Nominal data are presented in the form of quantitative values and percentages. The balance Hardy - Weinberg of the genotype distribution was checked using Online Encyclopedia for Genetic Epidemiology Studies (<http://www.oege.org/software/hwe-mr-calc.shtml>). To compare the distribution of genotypes in the experimental and control groups Pearson's chi-squared test was used. The reliability of differences of averages in groups with different genotypes was determined by the method of univariate analysis of variance (ANOVA). The impact of factors on the development of thyroid pathology was assessed using a binary logistic regression model for the relative risk (RelR), risk ratio (RR) and odds ratio (OR) with 95% confidence interval [95% CI], taking into account the criterion χ^2 (df = 1). The difference was considered reliable at $p < 0.05$.

Results and discussion

The best models of gene-gene interaction including those, where the TG function and the degree of its enlargement with the highest rates of cross-validation consistency are taken into account, are shown in table 2.

The method of multifactor dimension reduction (MDR) showed the reliability and a high reproducibility of a single-factor model with CTLA-4 gene involvement (table 2), both in the whole surveyed population of patients with risk of thyroid pathology persisted in one-component model involving the CTLA-4 gene as in euthyroidism (80% reproducibility, accuracy 69,27%, OR = 16,0; $p = 0.015$), somewhat stronger in subclinical and clinical hypothyroidism (100 % and 80% reproducibility, OR = 31,0; $p < 0.001$ and OR = 9,17; $p = 0.043$, respectively) and the second degree thyroid hyperplasia (100% reproducibility, OR = 6,37; $p = 0.022$).

In addition, this two-component model (CTLA-4 and Fas) proved to be effective for subclinical hypothyroidism (with the highest accuracy 97,41%; OR = 16,33; $p < 0.001$) and the I-degree thyroid hyperplasia (OR = 4,36; $p = 0.049$). The two-component model which involved the BCL-2 and CTLA-4 genes also confirmed its classification ability in the forecast of the II-degree thyroid hyperplasia (100% reproducibility; OR = 12,0; $p = 0.032$).

Graphic models of gene-gene interactions in thyroid pathology in the surveyed population (figures 1-3) show a high probability of euthyroidism with minor G-allele of the CTLA-4 and Bcl-2 (0,452 i 0,785 respectively) in the combination, which increases when AG-genotype of the Fas gene (1,452) is added to the combination.

Table 2

Models of gene-gene interactions among the surveyed individuals in general and considering thyroid gland pathology, the TG function and the degree of its enlargement

Groups	Combinations of genes in prognostic models	Model reproducibility	Testing cross-validation consistency	Model accuracy %	OR; p
euthyroidism	<i>CTLA-4</i>	8/10	4,26	69,27	<i>OR</i> =16,0; <i>p</i> =0,015
	<i>BCL-2, CTLA-4</i>	9/10	4,66	54,87	<i>OR</i> =2,71; <i>p</i> >0,05
	<i>BCL-2, CTLA-4, Fas</i>	10/10	5,51	58,65	<i>OR</i> =2,65; <i>p</i> >0,05
Subclinical hypothyroidism	<i>CTLA-4</i>	10/10	12,79	72,53	<i>OR</i> =31,0; <i>p</i> <0,001
	<i>CTLA-4, Fas</i>	7/10	12,64	97,41	<i>OR</i> =16,33; <i>p</i> <0,001
	<i>BCL-2, CTLA-4, Fas</i>	10/10	13,23	55,57	<i>OR</i> =3,12; <i>p</i> >0,05
Clinical hypothyroidism	<i>CTLA-4</i>	8/10	5,24	72,24	<i>OR</i> =9,17; <i>p</i> =0,043
	<i>BCL-2, CTLA-4</i>	8/10	4,63	64,60	<i>OR</i> =2,80; <i>p</i> >0,05
	<i>BCL-2, CTLA-4, Fas</i>	10/10	9,69	56,96	<i>OR</i> =1,29; <i>p</i> >0,05
I degree thyroid hyperplasia	<i>CTLA-4</i>	10/10	4,95	74,78	<i>OR</i> =6,37; <i>p</i> =0,022
	<i>CTLA-4, Fas</i>	10/10	17,33	48,21	<i>OR</i> =4,36; <i>p</i> =0,049
	<i>BCL-2, CTLA-4, Fas</i>	10/10	17,33	77,71	<i>OR</i> =1,17; <i>p</i> >0,05
II degree thyroid hyperplasia	<i>Fas</i>	5/10	2,79	51,47	<i>OR</i> =2,77; <i>p</i> >0,05
	<i>BCL-2, CTLA-4</i>	10/10	4,35	63,91	<i>OR</i> =12,0; <i>p</i> =0,032
	<i>BCL-2, CTLA-4, Fas</i>	10/10	4,35	61,24	<i>OR</i> =2,0; <i>p</i> >0,05

Note. TA – thyroid adenoma; AIT – autoimmune thyroiditis; TG – thyroid gland; *OR* - Odds Ratio.

The lowest probability of euthyroidism in thyroid pathology was observed in case of coincidence of wild alleles in the homozygous state of the three genes (-1.548) (fig. 3).

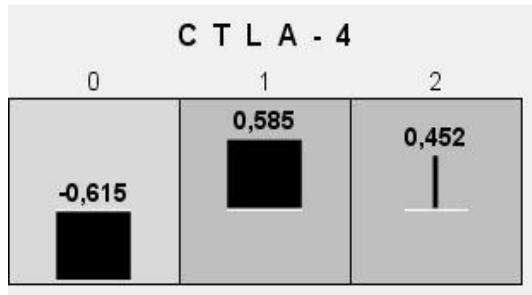


Fig. 1. Polymorphic variants of the *CTLA-4* gene (+49G/A), causing a high (dark grey box) and a low (light grey box) probability of euthyroidism in patients. Note: 0, 1, 2 – polymorphic variants of the *CTLA-4* gene- AA (0), AG (1), GG (2)

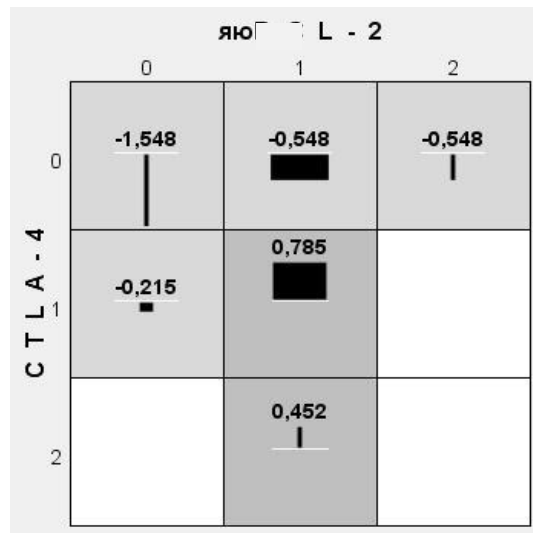


Fig. 2. Combination of polymorphic variants of *CTLA-4* (+49G/A) and *BCL-2* (63291411A>G) genes, causing a high (dark grey box) and a low (light grey box) probability of euthyroidism in thyroid pathology in patients. Note: *CTLA-4* 0, 1, 2 – polymorphic variants of the *CTLA-4* gene AA (0), AG (1), GG (2); 0, 1, 2 *Bcl-2* – 0, 1 – polymorphic variants of the *Bcl-2* gene AA (0), AG (1), GG (2). The white boxes mean the absence of the combinations of the gene genotypes.

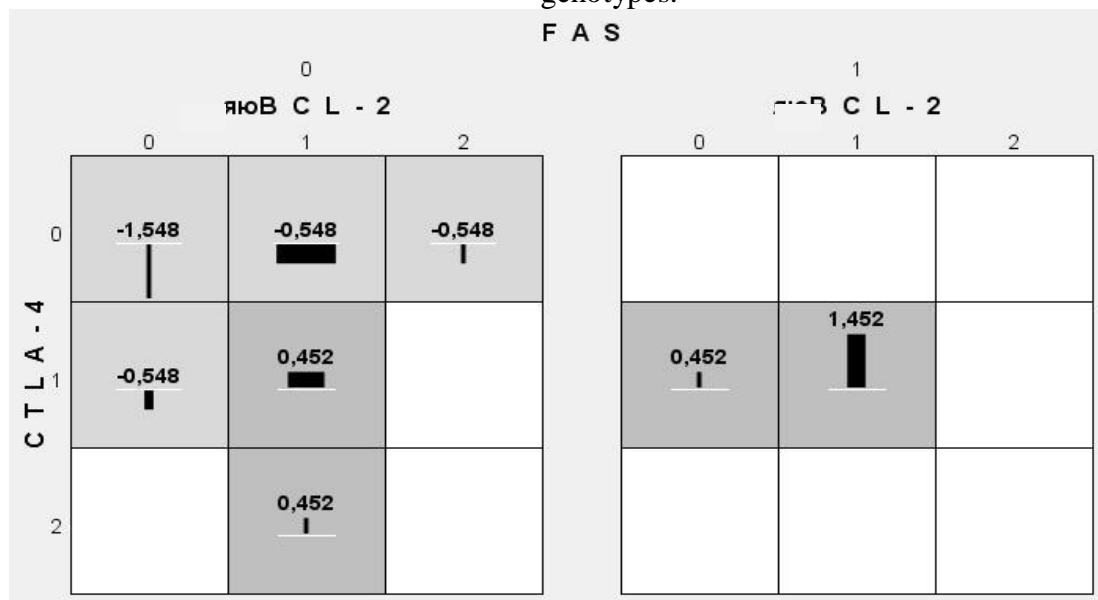


Fig. 3. Combination of polymorphic variants of *CTLA-4* (+49G/A), *Fas* (-1377G/A) and *BCL-2* (63291411A>G) genes, causing a high (dark grey box) and a low (light grey box) probability of euthyroidism in thyroid pathology in patients. Note: *CTLA-4* 0, 1, 2 – polymorphic variants of the *CTLA-4* gene AA (0), AG (1), GG (2); *Fas* 0, 1 – polymorphic variants of the *Fas* GG (0) gene, AG (1); 0, 1, 2 *Bcl-2* – polymorphic variants of the *Bcl-2* gene AA (0), AG (1), GG (2). The white boxes mean the absence of the combinations of the gene genotypes.

Analyzing the association of the gene polymorphic site with the condition of euthyroidism in patients suffering from thyroid pathology according to the entropy index showed that +49G/A polymorphic locus of the *CTLA-4* gene was the most influential (21,58%) (fig. 4). This effect was amplified with synergistic interaction with polymorphic site of the *Bcl-2* gene (63291411A> G) by 3.74%. The effects of polymorphisms of *Bcl-2* (rs17759659) and *Fas* (rs2234767) genes on euthyroidism onset in thyroid pathology were independent with entropy share 3,53% and 8,86% respectively.

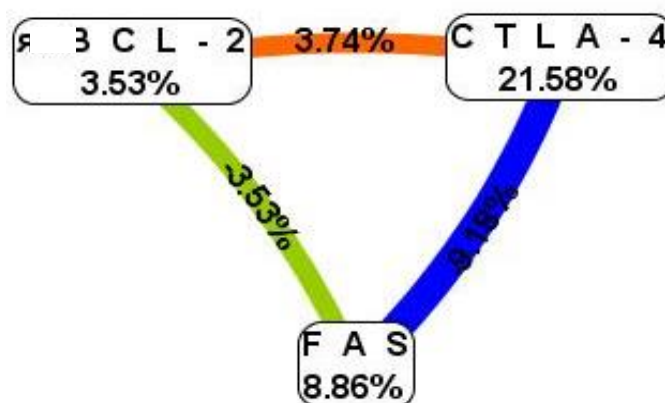


Fig. 4. Cluster analysis of modeling gene-gene interaction of three genes CTLA-4, Fas and Bcl-2 in patients with thyroid pathology in the condition of euthyroidism. The synergistic interaction is marked in orange, the independent impact is in green and blue.

In patients with thyroid pathology (fig. 5, 6) two-locus model involving AG-genotype (of *Fas* gene) and G-allele (of *CTLA-4* gene) and three-locus model involving AG-genotype (of *Fas* & *Bcl-2* genes) and G-allele (of *CTLA-4* gene) proved to be the best ones and increased the risk of subclinical hypothyroidism in the population of Northern Bukovina residents (1,115 and 1,282 respectively).

The risk of clinical hypothyroidism in thyroid pathology (fig. 7, 8) is the highest with three-locus combination of AG-genotype of the *CTLA-4* & *Fas* & *Bcl-2* genes (1,435), the two-locus model involving AG-genotype (of the *CTLA-4* and *Bcl-2* genes) proved to be weaker (0,685).

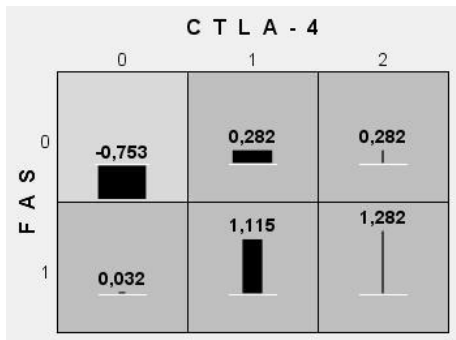


Fig. 5. Polymorphic variants of the *CTLA-4* (+49G/A) and *Fas* (-1377G/A) genes, causing a high (dark grey box) and a low (light grey box) risk of subclinical hypothyroidism in thyroid pathology in patients. Note: 0, 1, 2 – polymorphic variants of the *CTLA-4* gene- *AA* (0), *AG* (1), *GG* (2); *Fas* 0, 1 – polymorphic variants of the *Fas* *GG* (0) gene, *AG* (1).

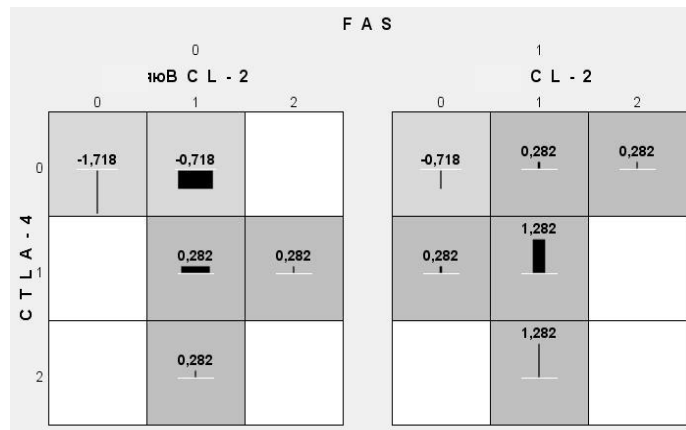


Fig. 6. Combination of polymorphic variants of the *CTLA-4* (+49G/A), *Fas* (-1377G/A) and *Bcl-2* (63291411A>G) genes, causing a high (dark grey box) and a low (light grey box) risk of subclinical hypothyroidism in thyroid pathology in patients. Note: *CTLA-4* 0, 1, 2 – polymorphic variants of the *CTLA-4* gene *AA* (0), *AG* (1), *GG* (2); *Fas* 0, 1 – polymorphic variants of the *Fas* gene *GG* (0), *AG* (1); 0, 1, 2 *Bcl-2* – polymorphic variants of the *Bcl-2* gene *AA* (0), *AG* (1), *GG* (2). The white boxes mean the absence of the combinations of the gene genotypes.

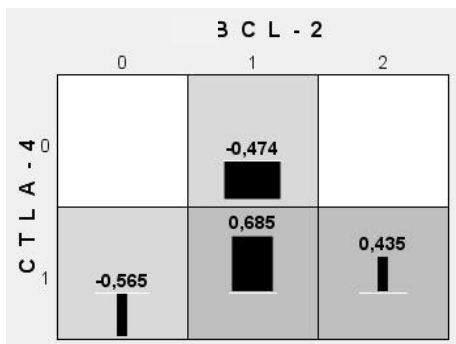


Fig. 7. Polymorphic variants of the *CTLA-4* (+49G/A) and *BCL-2* (63291411A>G) genes, causing a high (dark grey box) and a low (light grey box) risk of clinical hypothyroidism in thyroid pathology in patients. Note: 0, 1 – polymorphic variants of the *CTLA-4* gene- *AA* (0), *AG* (1); 0, 1, 2 *Bcl-2* – polymorphic variants of the *Bcl-2* gene *AA* (0), *AG* (1), *GG* (2). The white boxes mean the absence of the combinations of the gene genotypes.

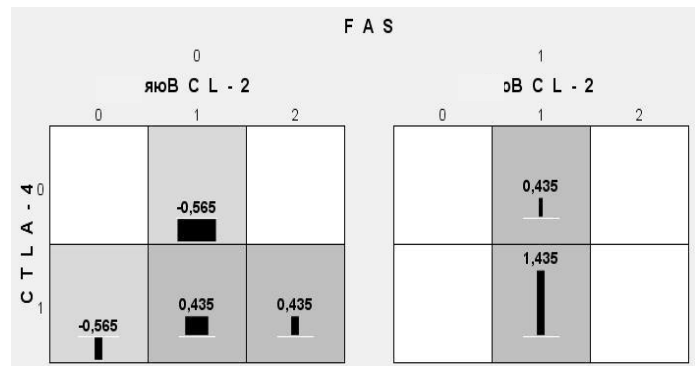


Fig. 8. Combination of polymorphic variants of the *CTLA-4* (+49G/A), *Fas* (-1377G/A) and *Bcl-2* (63291411A>G) genes, causing a high (dark grey box) and a low (light grey box) risk of clinical hypothyroidism in thyroid pathology in patients. Note: *CTLA-4* 0, 1 – polymorphic variants of the *CTLA-4* gene *AA* (0), *AG* (1); *Fas* 0, 1 – polymorphic variants of the *Fas* gene *GG* (0), *AG* (1); 0, 1, 2 *Bcl-2* – polymorphic variants of the *Bcl-2* gene *AA* (0), *AG* (1), *GG* (2). The white boxes mean the absence of the combinations of the gene genotypes.

According to the results of cluster analysis of modeling gene-gene interaction in subclinical and clinical hypothyroidism the highest share of entropy belonged to polymorphism of the *CTLA-4* (+49G/A) and *Fas* (-1377G/A) genes: 30,56% and 20,55% (fig. 9) and 13,67% and 6,17% (fig. 10), respectively. An independent connection between polymorphic loci of genes was found in subclinical hypothyroidism (from -13,05% to -1,61%) and synergistic one between polymorphic sites of the *CTLA-4* (+49G/A) and *Bcl-2* (63291411A>G) genes, which increased the risk of clinical hypothyroidism by 7,43% in the population (fig. 10).

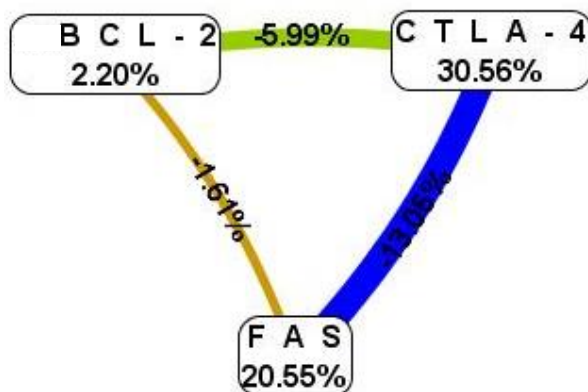


Fig. 9. Cluster analysis of modeling gene-gene interaction of three genes CTLA-4, Fas and Bcl-2 in patients with thyroid pathology in the condition of *subclinical hypothyroidism*. The independent effect is marked in green, brown and blue.

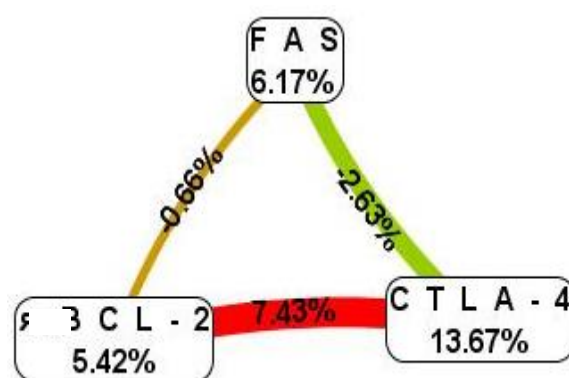


Fig. 10. Cluster analysis of modeling gene-gene interaction of three genes CTLA-4, Fas and Bcl-2 in patients with thyroid pathology in the condition of *clinical hypothyroidism*. The synergistic interaction is marked in red while the independent effect is in green and brown.

The risk of the I-degree thyroid hyperplasia in AIT and TA (fig. 11, 12) is the highest in three-locus combination of AG-genotype of the *CTLA-4* & *Fas* & *Bcl-2* genes (1,025; $p > 0,05$), the two-locus model involving AG- genotype of the *CTLA-4* & *Fas* genes is somewhat weaker but reliable (0,882; $p = 0,049$). The lowest is the risk of the I-degree thyroid hyperplasia in combination of wild alleles in the homozygous state of the *CTLA-4* & *Fas* genes (-0,975).

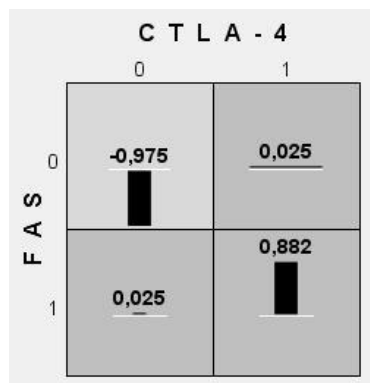


Fig. 11. Polymorphic variants of the CTLA-4 (+49G/A) and Fas (-1377G/A) genes, causing a high (dark grey box) and a low (light grey box) risk of *I-degree* thyroid hyperplasia in patients. Note: 0, 1 CTLA-4 – polymorphic variants of the CTLA-4 gene - AA (0), AG (1); 0, 1 Fas – polymorphic variants of the Fas GG (0) gene, AG (1).

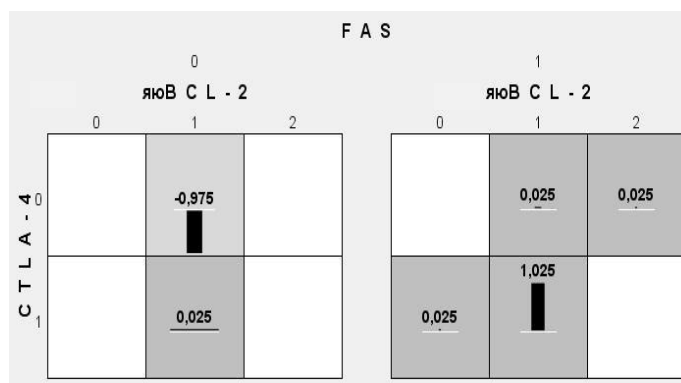


Fig. 12. Combination of polymorphic variants of the CTLA-4 (+49G/A), Fas (-1377G/A) and Bcl-2 (63291411A>G) genes, causing a high (dark grey box) and a low (light grey box) risk of *I-degree* thyroid hyperplasia in patients. Note: CTLA-4 0, 1, – polymorphic variants of the CTLA-4 gene AA (0), AG (1); Fas 0, 1 – polymorphic variants of the Fas gene GG (0), AG (1); 0, 1, 2 Bcl-2 – polymorphic variants of the Bcl-2 gene AA (0), AG (1), GG (2). The white boxes mean the absence of the combinations of the gene genotypes.

The risk of the II-degree thyroid hyperplasia in AIT and TA (fig. 13, 14) increases statistically significantly in two-locus combination of AG-genotype of the CTLA-4 & Bcl-2 genes (1,038; p=0,032). The three-locus model of gene-gene interaction involving AG-genotype of the three CTLA-4 & Fas & Bcl-2 genes proved to be stronger but unreliable (1,538). The lowest risk of I-degree thyroid hyperplasia was in the combination of wild alleles in homozygous state of both two genes CTLA-4 & Bcl-2 (-1,462), and in combination of three genes CTLA-4 & Bcl-2 & Fas (-1,462).

Cluster analysis of gene-gene interaction in *I-degree* thyroid hyperplasia (fig. 15) indicated the synergistic interconnection between polymorphic sites of the CTLA-4 and Fas genes with the highest entropy rate (10,07% and 7,15%), whereas in *II-degree* thyroid hyperplasia (fig. 16) – between polymorphic loci of the CTLA-4 and Bcl-2 genes (21,97% and 5,82% respectively). The entropy rate in the latter case was higher for gene Fas (10,47%), whose polymorphism influenced independently the onset of the *II-degree* thyroid hyperplasia in the population of surveyed residents of Bukovina (neutralizing effect on the impact of polymorphic site of the CTLA-4 gene was minus 11.53%; p> 0.05).

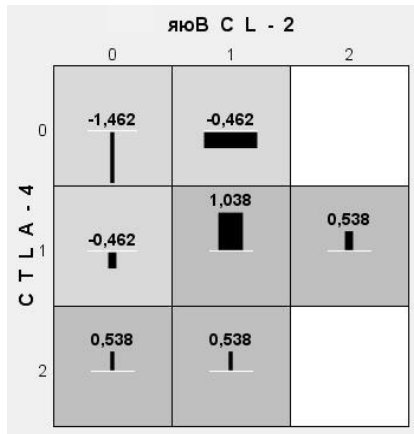


Fig. 13. Polymorphic variants of the CTLA-4 (+49G/A) and BCL-2 (63291411A>G) genes, causing a high (dark grey box) and a low (light grey box) risk of the *II-degree* thyroid hyperplasia in patients. Note: 0, 1, 2 – polymorphic variants of the CTLA-4 gene- AA (0), AG (1), GG (2); 0, 1, 2 Bcl-2 – polymorphic variants of the Bcl-2 gene AA (0), AG (1), GG (2).

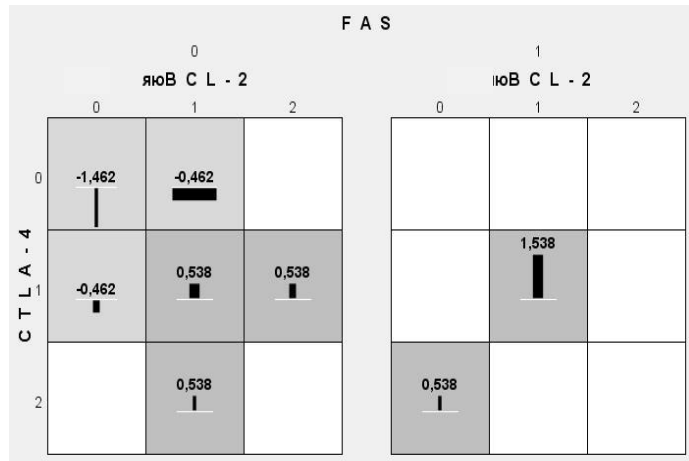


Fig. 14. Combination of polymorphic variants of the CTLA-4 (+49G/A), Fas (-1377G/A) and Bcl-2 (63291411A>G) genes, causing a high (dark grey box) and a low (light grey box) risk of *II-degree* thyroid hyperplasia in patients. Note: CTLA-4 0, 1, 2– polymorphic variants of the CTLA-4 gene AA (0), AG (1), GG (2); Fas 0, 1 – polymorphic variants of the Fas gene GG (0), AG (1); 0, 1, 2 Bcl-2 – polymorphic variants of the Bcl-2 gene AA (0), AG (1), GG (2). The white boxes mean the absence of the combinations of the gene genotypes.

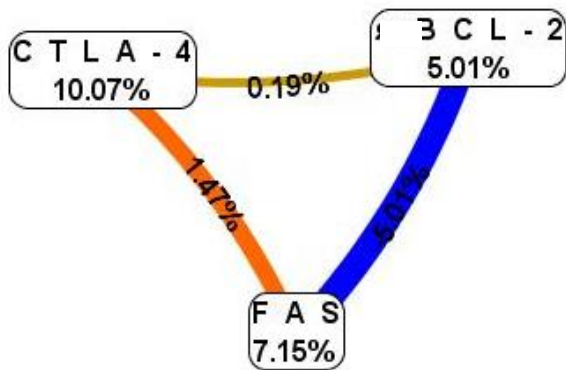


Fig. 15. Cluster analysis of modeling the gene-gene interaction of three genes: CTLA-4, Fas and Bcl-2 in patients with *I-degree* thyroid hyperplasia.

Note. The synergistic interaction is marked in orange, the independent effect is in blue and brown.

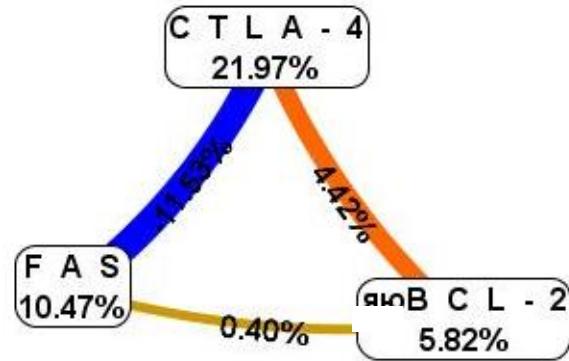


Fig. 16. Cluster analysis of modeling the gene-gene interaction of three genes: CTLA-4, Fas and Bcl-2 in patients with *II-degree* hyperplasia.

Conclusions: Graphic models of gene-gene interaction with the highest cross-validation consistency created by Multifactor Dimension Reduction 3.0.2 – MDR method, showed complex "synergistic or independent" impact of polymorphic loci of the CTLA-4 (+

49G / A), Fas (-1377G / A) and Bcl-2 (63291411A> G) genes on the onset of the change of thyroid function and degree of hyperplasia in the population of residents of Northern Bukovina.

The classification ability of the created models, despite the high reproducibility (60-100%), confirmed the likelihood of risks for a single-factor model involving the minor G-allele of the CTLA-4 gene (especially of AG-genotype) as well as for two-locus model in the combination of AG-genotype of the CTLA-4 and Fas genes: onset of condition of euthyroidism (exclusively with the participation of *CTLA-4* gene, subclinical hypothyroidism, clinical hypothyroidism, I-degree thyroid hyperplasia with the accuracy from 68,12% to 97,41% and testing Cross-validation Consistency from 4,26 to 20,55, respectively. The two-locus combination of AG-genotype of the CTLA-4 & Bcl-2 proved its effectiveness in the II-degree thyroid hyperplasia prognosis.

The three-component model involving three genes on the risk of thyroid pathology in the population in general CTLA-4 & Fas & Bcl-2 proved its classification capability. So did the combinations of:

- AG-genotypes of three genes which associates with the risk euthyroidism (1,452), clinical hypothyroidism (1,435), I-degree thyroid hyperplasia (1,025) and that of the II-degree (1,538);

- AG-genotypes of the Fas & *Bcl-2* genes and G-allele of the *CTLA-4* gene with the risk of AIT (1,392) and subclinical hypothyroidism (1,282).

Interlocus interaction in thyroid pathology is characterized by pronounced synergistic gene-gene relationship (*CTLA-4* & *Fas*, *CTLA-4* & *Bcl-2* from 1,47% to 7,43%), or by independent, neutralizing effect (from -13,05% to 0,35%) with the highest entropy rate which is typical for *CTLA-4* gene (21,58% – in euthyroidism, 30,56% – in subclinical hypothyroidism, and 13,67% – in clinical hypothyroidism), which indicates its high contribution to the development of thyroid pathology both in general (21,38%), and of some of its nosology's. Somewhat less significant (by 1,5-5,5 times) is the share in the development of thyroid pathology concerning the entropy rate made by the *Fas* gene (3,86%): 8,99% – in TA, 12,14% – in AIT, 8,86% – in euthyroidism, 20,55% – in subclinical hypothyroidism, 6,17% – in clinical hypothyroidism, respectively.

Conflict of interest. The authors declare no conflict of interest concerning this article.

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References

1. Dong YH, Fu DG. Autoimmune thyroid disease: mechanism, genetics and current knowledge. *Eur Rev Med Pharmacol Sci.* 2014;18(23):3611-3618.
2. Tomer Y, Davies TF. Searching for the autoimmune thyroid disease susceptibility genes: from gene mapping to gene function. *Endocr Rev.* 2003;24(5):694-717.
3. Brix TH, Hegedus L. Twin studies as a model for exploring the aetiology of autoimmune thyroid disease. *Clin Endocrinol (Oxf).* 2012;76(4):457-464.
4. Lee Y.H, Choi S.J., Ji J.D., Song GG. CTLA-4 and TNF- α promoter-308 A/G polymorphisms and ANCA-associated vasculitis susceptibility: a meta-analysis// *Mol Biol Rep.* 2012 Jan, 39 (1):319-326.
5. Zhang M, Ni J, Xu WD et al. Association of CTLA-4 variants with susceptibility to inflammatory bowel disease: a meta-analysis// *Hum Immunol.* 2014; 75(3): 227-233.
6. Camilot M, Teofoli F, Gandini A, Franceschi R, Rapa A, Corrias A, Bona G, Radetti G, Tatò L 2005 Thyrotropin receptor gene mutations and TSH resistance: variable expressivity in the heterozygotes. *Clin Endocrinol (Oxf)* 63:146–151
7. Tonacchera M, Di Cosmo C, De Marco G, Agretti P, Banco M, Perri A, Gianetti E, Montanelli L, Vitti P, Pinchera A 2007 Identification of TSH receptor mutations in three families with resistance to TSH. *Clin Endocrinol (Oxf)* 67:712–718.
8. Qiu H., Tang W., Yin P., Cheng F., Wang L. Cytotoxic T-lymphocyte associated antigen 4 polymorphism and Hashimoto's thyroiditis susceptibility: a meta-analysis // *Endocrine.* 2014; 45(2):198-205.
9. Pastuszek-Lewandoska D., Sewerynek E., Domańska D. et al. CTLA-4 gene poly-morphisms and their influence on predisposition to autoimmune thyroid diseases (Graves' disease and Hashimoto's thyroiditis) // *Arch Med Sci.* 2012 Jul 4, 8(3):415-421.
10. Sheremet MI, Sydoruk LP, Shidlovskiy VO, Bedenyuk AD. et al. Effect of APO-1 / FAS, CTLA-4 AND BCL-2 genes polymorphisms on the risk of goiter nodular forms with NGAIT occurrence among the Bukovinian population. *Archives of the Balkan Medical Union* 2017; 52(2):144-151.
11. Effraimidis G., Strieder T.G., Tijssen J.G., Wiersinga WM. Natural history of the transition from euthyroidism to overt autoimmune hypo- or hyperthyroidism: a prospective study// *Eur J Endocrinol,* 2011; 164:107-113.

12. Mazziotti G., Sorvillo F., Naclerio C. et al. Type-1 response in peripheral CD4+ and CD8+ T cells from patients with Hashimoto's thyroiditis // *Eur J.Endocrinol.* 2003; 148:383–388.
13. Leithäuser F, Dhein J, Mechtersheimer G, et al: Constitutive and induced expression of APO-1, a new member of the nerve growth factor/tumor necrosis factor receptor superfamily, in normal and neoplastic cells. *Lab Invest.* 1993. 69: 415-429.
14. Man-Man Lu, Qian-Ling Ye, Chen-Chen Feng, *et al*: Association of FAS gene polymorphisms with systemic lupus erythematosus: A case-control study and meta-analysis. *Experimental and Therapeutic Medicine.* 2012; 4: 497-502.
15. Gözü H.I. Is the TSHR D727E polymorphism a genetic predisposition for multinodular goiter in the Turkish population? / H.I. Gözü, S. Özçelik, M. Aloğlu, A. Şahin, S. Temiz, A. Dayan, H. Cengiz, Y. Tütüncü, R. Bircan // *Genet Mol Res.* 2016; 15(3):385–90.
16. Kochetova O.V. DIO2, TPO, CYP1A1 AND CYP1A2 gene polymorphism in women with thyroid disease. / Kochetova O.V., Gaynullina M.K., Viktorova T.V. // *Gig Sanit.* 2014; 3:52–56.