Nat. Volatiles & Essent. Oils, 2021; 8(5): 12496-12500

NVEO
Natural Volatiles & Essential Oils

DISTRIBUTION OF ALLELES AND GENOTYPES OF CTLA4 AND TNFA POLYMORPHISM IN PATIENTS WITH AUTOIMMUNE THYROIDITIS

Rana Rahimova[1], Gulnara Dashdamirova[2], Sabina Guliyeva[3]
[1,2,3]Azerbaijan Medical University

The present study aimed to examine the frequency of alleles and genotypes of CTLA4 and TNFα gene polymorphism in patients with autoimmune thyroiditis.
Under supervision were 170 patients with autoimmune thyroiditis (AIT), 64 men and 106 women, aged 18 to 64 years. The control group was 65 people without thyroid pathologies and other autoimmune diseases aged 20 to 65 years, of which 26 were men and 39 were women.
Genotyping of single nucleotide polymorphisms CTLA-4 and TNF-α was carried out based on the genetic laboratory of the YASHAM clinic.
For molecular genetic analysis on polymorphisms of loci CTLA4 (rs231775) and TNF, real-time PCR was performed with fluorescently labeled probes on the CFX96 (BioRad) instrument, followed by visualization and interpretation of the results in the Bio-Rad CFX-96 program.
Statistical analyses were performed using Statistica 12, Microsoft Office Excel, and Microsoft Office Word 2010.
Analysis of the distribution of allele frequencies and polymorphism genotypes rs 231775 of the CTLA-4 gene among AIT patients revealed a statistically significant increase in the occurrence of the "rare" G allele (48%) compared to control group (33.8%), $p < 0.05$ ($\chi 2 = 4.27$, OR = 1.865, 95% CI = 1.028-3.382) and a decrease in allele A, (51.2%) relative to control group (66.1%), $p < 0.05$ ($\chi 2 = 4.27$, OR = 0,536, CI = 0,296,-,0,973).
Carriers of the G allele and the GG genotype have an increased risk of developing AIT, while carriers of the A allele have a reduced developing risk of this disease. The G allele and AG, GG genotypes of the TNF-α gene 308AG polymorphism are risk factors for the development of AIT and therefore genetic markers of AIT. Analysis of the frequency of occurrence of allele A and genotype AA showed a statistically significant increase in both study parameters in individuals in the control group, 73.8% and 64.6% compared to patients with AIT, in whom these values were 57.1%, respectively, for allele A ($p = 0.0179$, $\chi 2 = 5.61$, OR = 0.471, 95% CI = 0.250 - 0.885) and 24.1%, for the AA genotype ($p = 0.0000$, $\chi 2 = 33.76$, OR = 0.174, 95% CI = 0.094-0.323).

Keywords: autoimmune thyroiditis (AIT), Hashimoto's thyroiditis, allele, genotype, polymorphism, CTLA-4 gene, TNFα gene, marker.

Introduction

Autoimmune thyroiditis is a group of organ-specific autoimmune thyroopathies caused by a genetically determined defect in immune tolerance to thyroid antigens, resulting in its autoimmune damage.

Genetic factors play an important role in the development of TD, which has been repeatedly demonstrated in epidemiological studies. This is evidenced by:
- the family nature of AIT (in 20-30% of cases, the disease develops in the siblings of patients, the value of the odds ratio (OR) is 16;
- high AIT concordance in monozygous twins (29-55%, in dizygotic twins - 0-7%);
- the presence of circulating ATA in about 50% of patient siblings [Tomer Y., Davies T. F., 2013; P. 695].

TD is markedly more commonly associated with such diseases, especially Addison's disease, and type I diabetes mellitus, which may result from the influence of genetic and/or environmental factors [Wiebolt J. et al., 2011; P.792]. A recent study of Han multiplex families in China showed a genetic predisposition to inherit autoantibody patterns to thyroid gland tissue [Hou X. et al., 2011; P.1358]. Several studies conducted by Chinese, Japanese researchers have shown that the presence of certain genes in humans is associated with an increased risk of TD [Huang C.Y. et al., 2012; P.436, Ueda S. et al., 2014; E383].

When examining a sample of 444 Japanese patients with TD, it was determined that haplotypes HLA-A*02:07 and HLA-DRB4 predispose to the development of TD, while haplotypes HLA-A*33:03-C*14:03-B*44: 03-DRB1* 13:02-DQBB*06:04-DPB1*04:01, in contrast, "protect" against the manifestation of this disease [Ueda S. et al., 2014; E383].

The purpose of the study

The present study aimed to examine the frequency of alleles and genotypes of CTLA4 and TNFα gene polymorphism in patients with autoimmune thyroiditis

Research materials and methods.

Under supervision were 170 patients with autoimmune thyroiditis (AIT), 64 men and 106 women, aged 18 to 64 years. The control group was 65 people without thyroid pathologies and other autoimmune diseases aged 20 to 65 years, of which 26 were men and 39 were women.

It should be noted that the observed distribution of genotype frequencies over the studied locus of the studied genes in the control sample corresponds to the theoretically expected Hardy-Weinberg equilibrium distribution (p = 0.76).

Diagnosis of AIT was established based on data from history, thyroid status, results of thyroid ultrasound (thyroid gland), positive antibodies to the thyroid hormone receptor (TSH). The diagnosis of the manifest form of AIT was made based on the clinical picture of the disease, that is, the patient's complaints (obesity, reduced body temperature, as well as toughness - the permanent feeling of cold due to worsening metabolism and yellowness of the skin, myxedematous edema - circles under the eyes, difficulty breathing and worsening of hearing, change of voice, drowsiness, stagnation of processes such as thinking, speech, emotionality, shortness of breath, painful sensations in the heart and beyond the sternum area, reduction of heart rate or heart enlargement, the tendency to constipation, diarrhea, and liver enlargement, loss of limb sensitivity, thinning and hair loss, disturbance or cessation of menstruation), higher TSH level and lower levels of hormones T3 and T4, as well as increased antibody titers in TSH - ATTG, ATPO. The diagnosis of the subclinical form of AIT was made based on an increase in TSH levels and normal values of the hormones T3 and T4. The clinical picture in this form of the disease is erased. Genotyping of single nucleotide polymorphisms CTLA-4 and TNF-α was carried out based on the genetic laboratory of the “YASHAM” clinic. DNA isolation from whole venous blood using the reagent "DNA - EXPRESS - BLOOD" (NPF Litech, Russia).

The collection of biological material (peripheral venous blood samples) was carried out by medical workers based on three institutions: the therapeutic clinic of Azerbaijan Medical University, the Department of Biochemistry of Azerbaijan Medical University and the medical center "YASHAM" (Baku).

Total genomic DNA was isolated by phenol-chloroform extraction. The selection of markers for analysis is due to functions of gene products - transcription factors, cytokines, and their receptors. For molecular genetic analysis on polymorphisms of loci CTLA4 and TNF, real-time PCR was performed with fluorescently labeled probes on the CFX96 (BioRad) instrument, followed by visualization and interpretation of the results in the Bio-Rad CFX-96 program.

Statistical analyses were performed using Statistica 12, Microsoft Office Excel, and Microsoft Office Word 2010.

Results of the study.

The results of the study of frequencies of polymorphic variants of the CTLA-4 gene in a sample of patients with AT are presented in Table 1.

Analysis of the distribution of allele frequencies and polymorphism genotypes rs 231775 of the CTLA-4 gene among AIT patients revealed a statistically significant increase in the occurrence of the "rare" G allele (48%) compared to control group (33.8%), $p < 0.05$ ($\chi 2 = 4.27$, OR = 1.865, 95% CI = 1.028-3.382) and a decrease in allele A, (51.2%) relative to control group (66.1%), $p < 0.05$ ($\chi 2 = 4.27$, OR = 0,536, CI = 0,296,-,0,973).

In the group of patients with AIT, a significant increase in the frequency of the homozygous genotype GG by the polymorphic locus of the CTLA-4 gene was also shown (25.9%) compared to the control group, where the relative frequency of this genotype was 10.8%, ($\chi 2\ 2 = 6.32$, OR = 2.893, 95% CI = 1.229-6.811.

For the other two polymorphic variants of genotypes - AA and AG of the CTLA-4 gene in patients with AIT there are no reliable differences in the frequency of occurrence relative to the persons of the control group, $p > 0.05$.

So, the G allele and the GG genotype of the polymorphic marker + 49A/G gene CTLA4 prevailed in the groups of patients with AIT, at the same time, the frequency of allele A in healthy persons significantly exceeded the frequency of allele A in the group of patients with AIT. The differences identified are highly reliable. AA and AG genotypes occurred more frequently in the control group relative to AIT patients, but the difference identified was not statistically reliable.

We can conclude that carriers of the G allele and the GG genotype have an increased risk of developing AIT, while carriers of the A allele have a reduced risk of developing this disease. The obtained results show that the polymorphic marker + 49A/G of the CTLA4 gene is closely associated with the predisposition to AT in patients in the Azerbaijani population.

Table 1

Distribution of allele frequencies and polymorphism genotypes + 49A/G (rs 231775) of the CTLA-4 gene in patients with AIT and in the control group

Alleles and genotypes	Patients with AIT with an outcome in hypothyroidism n = 170		Control group n = 65		P	χ^2	OR	95% CI
	n	%	n	%				
AA	53	31.2	24	36.9	>0,05	-	-	-
AG	73	42.9	34	52.3	>0,05	-	-	-
GG	44	25.9	7	10.8	P=0.0119	6.32	2.893	1.229-6.811
A	87	51.2	43	66.1	P=0.0389	4.27	0.536	0.296-0.973
G	83	48.8	22	33.8	P=0.039	4.27	1.865	1.028-3.382

The TNFα gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. This cytokine is mainly secreted by macrophages. TNFα plays an important role in initiating an adaptive immune response. TNFα is produced by monocytes, T cells, natural killers, and mast cells. It is involved in the upregulation of class I HLA, phagocyte activation, induction of IL1 itself, IL8 and TNFα, and, synergistically with IFNγ, in enhancing HLA class II expression. TNFα is found in thyroid tissue in Graves' disease and Hashimoto thyroiditis patients at higher levels than in healthy individuals. It can bind and, act through its receptors TNFRSF1A/ TNFR1 and TNFRSF1B/TNFBR. This cytokine is involved in the regulation of a wide range of biological processes, including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. It is involved in many diseases, including autoimmune diseases. When analyzing the distribution of allele frequencies and genotypes of the 308 AG polymorphism of the TNF-α gene, significant differences of this marker between the test groups were established in the examined persons (Table 2).

Studies have shown significantly high values of the G allele, AG and GG genotypes of the polymorphism 308AG the TNF-α gene in patients with AIT. Thus, the G allele was detected in 49.2% of patients with AIT, which is significantly higher than in those in the control group, P = 0.0179 (26.1%), ($\chi 2$ = 561, OR = 2.125, 95% CI = 1.131-3.994). As mentioned above, in patients with AIT, the genotypes of GG and AG polymorphism 308AG are observed significantly more often, respectively, in 20% and 55.9% in comparison with similar indicators in the control group, 4.6% (p = 0,0038, $\chi 2$= 8.39, OR = 5,176,,95% CI = 1.52-17.46) and 30.7%, respectively, (p = 0,0006, $\chi 2$= 11.87, OR = 2.85. 95% CI = 1.55-5.23) As can be seen from the table below, the G allele, AG and GG genotypes of the polymorphism of the TNF-α gene 308AG are risk factors for the development of AIT and therefore genetic markers of AIT. Analysis of the incidence of allele A and genotype AA showed a statistically significant increase in both study measures in individuals in

the control group, 73.8% and 64.6% compared to patients with AIT, in whom these values were 57.1%, respectively, for allele A (p = 0.0179, χ2 = 5.61, OR = 0.471, 95% CI = 0.250 - 0.885) and 24.1%, for the AA genotype (p = 0.0000, χ2 = 33.76, OR = 0.174, 95% CI = 0.094-0.323).

It follows from the above that these two markers: allele A and homozygous genotype AA are protective concerning the development of AIT in individuals of the Azerbaijani population.

So, our study reports a statistically significant association between the G allele and the GG, AG genotypes of the TNFα 308AG gene polymorphism in AIT.

Table 2

Allele frequency distribution and polymorphism genotypes 308AG TNF-α gene in AIT patients and control group

Alleles and genotypes	Patients with AIT with an outcome in hypothyroidism n = 170		Control group n = 65		P	χ^2	OR	95% CI
	n	%	n	%				
AA	41	24.1	42	64.6	P=0.000	33.76	0.174	0.094-0.323
AG	95	55.9	20	30.7	P=0.0006	11.87	2.85	1.55-5.23
GG	34	20.0	3	4.6	P=0.0038	8.39	5.176	1.52-17.46
A	97	57.1	48	73.8	P=0.0179	5.61	0.471	0.250-0.885
G	73	49.2	17	26.1	P=0.0179	5.61	2.125	1.131-3.994

Discussion of the results.

Autoimmune diseases are multifactorial diseases in which the combination of genetic predisposition with the adverse effect of environmental factors triggers a cascade of reactions in the immune system leading to auto aggression with damage to thyroid tissue. Genetic predisposition plays a defining role in the mechanisms of manifestation and progression of autoimmune inflammation in autoimmune-derived thyroiditis. In the present work, we investigated the detection of the frequency of occurrence of genotypes and alleles of CTLA-4 and TNF-α genes in patients with AIT.

As known, the greatest contribution to the predisposition to autoimmune thyroid diseases belongs to the IDDM1 locus, where the genes of the main histocompatibility complex of HLA class II are located.

Studies showed that in patients of Azerbaijani nationality with AIT there is a statistically significant increase in the frequency of the homozygous genotype GG by the polymorphic locus of the CTLA-4 gene, (25.9%) in comparison with the control group, where the relative frequency of this genotype is 10.8%, (χ2 = 6.32, OR = 2.893, 95% CI = 1.229-6.811).

An allele study of the above gene in patients with AIT established an increase in the occurrence of the "rare" G allele (48%) compared to control group (33.8%), $p < 0.05$ (χ2= 4.27, OR = 1.865, 95% CI = 1.028-3.382) and a decrease in allele A, (51.2%) relative to control group (66.1%), $p < 0.05$ (χ2= 4.27, OR = 0,536, CI = 0,296,-,0,973). World literature data on the role of genetic markers in the manifestation of AIT in patients with different genotypes are few and contradictory [Niyazoglu M, Baykara O, Koc A, 2014; 547 (2): 226-232, Allahabadia A, Heward JM, Nithiyananthan R. 2001;358(9286):984-985, Bednarczuk T, Hiromatsu Y, Fukutani T., Eur J Endocrinol. 2003;148:13-18]. This is due to both different ethnicity and differences in criteria for inclusion in study groups. In addition, multifactorial diseases, which include AIT, are characterized by the realization of a genetic predisposition to the disease only in the presence of certain combinations of genotypes, alleles, and trigger environmental factors. Therefore, the present paper examined the frequency of occurrence of the genotype and allele of the TNF-α gene.

In our study, we used a polymorphic marker 308AG the TNF-α gene, which is in partial disequilibrium by adhesion to the TNF gene marker 238AG (rs361525) in the EuropeAid population. Examination of the frequency of distribution of genotypes and alleles of the TNF-α gene revealed an increase in the frequency of the G allele and the AG, GG genotypes of the TNF-α 308AG polymorphism in patients with AIT.

Genotypes of GG and AG polymorphism 308AG in AIT are observed respectively in 20% and 55.9%, and in the control group, 4.6% (p = 0,0038, χ2 = 8.39, OR = 5,176,,95% CI = 1.52-17.46) and 30.7%, respectively, (p = 0,0006, χ2 = 11.87, OR = 2.85. 95%, CI = 1.55-5.23). A significant difference in the frequency of these genotypes is noticeable. A significant difference between the comparison groups was also noted in the incidence of the G. allele. Thus, the G allele was detected in 49.2% of patients with AIT, which is significantly higher than in those in the control group, P = 0.0179 (26.1%), (χ2 = 561, OR = 2.125, 95% CI = 1.131-3.994).

The incidence of allele A and genotype AA was significantly higher in individuals in the control group, 73.8% and 64.6% compared to patients with AIT, in whom these values were 57.1%, respectively, for allele A (p = 0.0179, χ2 = 5.61, OR = 0.471, 95% CI = 0.250-0.885) and 24.1%, for the AA genotype (p = 0.0000, χ2= 33.76, OR = 0.174, 95% CI = 0.094-0.323).

It follows from the above that these two markers: allele A and homozygous genotype AA are protective markers concerning the development of AIT in individuals of the Azerbaijani population.

So, our study reports a statistically significant association between the G allele and the GG, AG genotypes of the TNFα 308AG gene polymorphism in AIT patients. This is consistent with studies by several authors that have identified an association between these markers and Graves' disease, suggesting that the allele may play a role in the pathogenesis of AIT. But contradictory data on genotypic and allelic frequencies of polymorphism 308AG TNFα in GD and Hashimoto thyroiditis have also been found in the literature.

The TNFα gene is located in the HLA class III domain of the main histocompatibility complex (MHC). Studies are showing the genetic contribution of HLA regions to susceptibility to AIT. HLAs are regions of strong nonequilibrium adhesion, so it cannot be ruled out that the association of TNFα variants with GD and HD may not be associated with polymorphism within the TNFα gene itself, but with a change in the linked gene.

References

1. Tomer Y., Davies T.F. Searching for the autoimmune thyroid disease susceptibility genes: from gene mapping to gene function // Endocrine Reviews. – 2013. – Vol. 24. – P. 694-717.
2. Wiebolt J., Achterbergh R., den B.A. et al. Clustering of additional autoimmunity behaves differently in Hashimoto's patients compared with Graves 'patients // Eur. J. Endocrinol. – 2011. – Vol. 164. – P. 789–794.
3. Hou X., Li Y., Li J. Development of thyroid dysfunction and autoantibodies in Graves 'multiplex families: an eight-year follow-up study in Chinese Han pedigrees // Thyroid. – 2011. – Vol. 21. – P. 1353–1358.
4. Huang C.Y., Chang T.Y., Chu C.C. et al. The HLA-B gene and Hashimoto disease in Han Chinese children: a case-control and familybased study // Tissue Antigens. – 2012. – Vol. 80. – P. 431–436.
5. Ueda S., Oryoji D., Yamamoto K. et al. Identification of independent susceptible and protective HLA alleles in Japanese autoimmune thyroid disease and their epistasis // J. Clin. Endocrinol. Metab. – 2014. – Vol. 99. – P. 379–383.
6. Bednarczuk T, Hiromatsu Y, Fukutani T, Jazdzewski K, Miskiewicz P, Osikowska M, Nauman J. Association of cytotoxic T lymphocyte- associated antigen-4 (CTLA-4) gene polymorphism and non-genetic factors with Graves' ophthalmopathy in European and Japanese populations. Eur J Endocrinol. 2003;148:13-18.
7. Allahabadia A, Heward JM, Nithiyananthan R, Gibson SM, ReuserTT, Dodson PM, Franklyn JA, Gough SCL. MHC class II region, CTLA-4 gene, and ophthalmopathy in patients with Graves' disease. Lancet. 2001;358(9286):984-985.
8. Niyazoglu M, Baykara O, Koc A, Aydoğdu P, Onaran I, Dellal FD, Tasan E, Sultuybek GK. Association of PARP-1, NF-κB, NF-κBIA and IL-6, IL-1β and TNF-α with Graves Disease and Graves Ophthalmopathy. Gene. 2014;547(2):226-232.

Disclosure of conflicts of interest. The author declares that she has no financial or other potential conflicts of interest.