Association of polymorphism of HLA II genes with chronic adrenal insufficiency in APS 2,3,4 types – protective and predisposing genes.

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Materials and methods

The case-control study involved 78 patients with APS 2, 3, 4 types and 109 healthy subjects. Alleles of the HLA II class genes, CTLA4 and PTPN22 were identified by the multiprimer allele-specific PCR method. The statistical analysis was carried out using the exact two-sided Fisher test. The association of the chronic adrenal insufficiency in patients with APS was determined by the value of the odds ratio (OR - odd's ratio), the value of 95% confidence interval (95% CI).

Results

Haplotypes DR3-DQ2 (OR = 4.06), DR4-DQ8 (OR = 5.78), genotype DR3/DR4 (OR = 19.7), DQA1*0301 allele (OR = 4.27), as well as genotype DQA1*0301/DQA1*0501 (OR = 13.89) predispose to the development of APS type 2, 3 and 4 in adults compared to the control group. APS patients were divided into two groups according to the presence of Addison’s disease (APS 2 and 4 types - and type 3 APS). Haplotype DR3-DQ2 (DRB1*17-DQA1*0501-DQB1*0201) (OR = 2.6), as well as the genotype DR3/DR4 (OR = 4.28) found the strongest association with the development of adrenal insufficiency in patients with APS.

Haplotype DRB1*01-DQA1*0101-DQB1*0501 (OR = 0.07), as well as DRB1*01 (OR = 0.08) have been determined as protective for the development of Addison’s disease.

Conclusion

Examination of APS type 3 patients without Addison’s disease for the presence of protective genes for the development of adrenal insufficiency will allow better predicting the risk of developing of the disease within the syndrome.

Introduction

Autoimmune polyglandular syndromes (APS) are the combinations of a variety of autoimmune endocrine and non-endocrine diseases represented by the two main types – APS type 1 and APS type 2. Another two types - APS type 3 and APS type 4 are relating to APS of adults according to classification of clinical character [1, 5].

Nowadays in Russian practice there is no enough focus on diagnostic risks of development the new components of syndrome in APS type 3 patients. Such patients have a combination of autoimmune thyroid disorders with endocrine and non-endocrine autoimmune diseases – Type 1 Diabetes or LADA, vitiligo, alopecia, coeliacia, autoimmune atrophic gastritis, systemic lupus erythematosus, etc. excluding Addison’s disease.

The regular examination for the autoimmune markers of the new components of APS, and also revealing of predispositional and protective genes for Addison’s disease (HLA DR3, DR4) allow to predict the risk of sudden onset of complications within the syndrome (adrenal crisis and heavy forms of hypoglycemia at the onset of chronic adrenal insufficiency) [6].

The Aim of the study was to determine the association of chronic adrenal insufficiency with polymorphism of HLA II genes among patients with APS 2,3,4 types. The focus of the study was on the revealing of protective genes for Addison’s disease in APS 3 type patients.
Materials and methods

Sera of 78 patients with APS type 2, 3, 4 and 109 healthy subjects were screened for HLA II genes’ polymorphism. APS patients were at the age of 18-78 years, women – 74,4 %, men— 25,6 %. The control healthy group was at the age of 18-58, among them women - 64,2 %, men - 35,8 %. The difference between two groups was not statistically significant on gender (p=0,154).

Molecular-genetic examination of HLA II genes was conducted for all patients. DNA from the whole blood of the person was carried out by set of QIAamp DNA Blood Mini Kit (QIAGEN). The method of multiprimer allele-specified polymerase chain reaction was used.

Statistical analysis was carried out by STATISTICA 10, SPSS Statistics with the use of precise two-sided Fisher’s test. The differences were considered significant with p<0,05. Association with the disease was determined by OR – odd’s ratio and 95% of confidence interval (95 % CI). The strong association with the development of the disease was set up with the meaning OR and 95% CI. The results of the research are similar to the results of international studies, conducted among different populations. According to Norwegian and Italian research the frequency of chronic adrenal insufficiency in APS type 2 and 4 with chronic adrenal insufficiency (N=36) and APS type 3 without chronic adrenal insufficiency (N=42). The two groups had significantly differences in the frequency of occurrence of vitiligo – 6 % against 17% in accordance (p =1,0).

The prevalence of vitiligo in adult APS patients was 11,5 %, alopecia - 5,1 %, celiac disease – 2,56 %.

APS patients were separated into two groups- APS type 2 and 4 with chronic adrenal insufficiency (N=36) and APS type 3 without chronic adrenal insufficiency (N=42). The two groups had significantly differences in the frequency of occurrence of vitiligo – 6 % against 17% in accordance (p =1,0).

The frequencies of predisposing and protective HLA II alleles in the group of APS of adults and control group are represented in the Table 1.

### Results

Addison’s disease occurred in 46,2 % cases of APS patients, autoimmune thyroid disorders – in 89,7 %.

- Chronic autoimmune thyroiditis was represented at 84,3 %, Graves’ disease had 15,7 % of patients. Autoantibodies to the thyroid tissue (anti-TPO and anti-TG) without thyroid dysfunction were elicited in 5,1% cases of patients.
- Type 1 Diabetes was determined in 56,4 % cases, among them LADA – in 15,9 %. The positive level of diabetes-associated autoantibodies (ICA, IA 2, GAD) without increase of glucose level was determined in 7,7 % among all the APS patients.
- The prevalence of vitiligo in adult APS patients was 11,5 %, alopecia - 5,1 %, celiac disease – 2,56 %.

Comparing the two groups of patients with APS 2,3,4 types and patients from the control group the association of haplotypes DR3-DQ2 (DRB1*0301-DQA1*0501-DQB1*0201), DR4-DQ8 (DRB1*04-DQA1*0301-DQB1*0302), and also genotype DR3-DQ2/DR4-DQ8 was elicited with the development of the syndrome (Fisher exact, two-tailed - p < 0.0001; OR=4.0609, 95 % CI [2.0955 - 7.8695], OR=5.7815 95 %, CI [3.1380 - 10.6520] and OR=19.7105, 95 % CI [4.4618 - 87.0743] in accordance.

The results of the research are similar to the results of international studies, conducted among different populations. According to Norwegian and Italian research the frequency of chronic adrenal insufficiency in APS type 2 significantly increased with the occurrence of haplotypes DR3-DQ2 and DR4-DQ8 [2, 6, 8].

In the Table 2 there are frequencies of predisposing and protective haplotypes of HLA II genes for APS type 2, 4 and APS type 3.
Comparing APS type 2, 4 (with chronic adrenal insufficiency) and APS type 3 (without chronic adrenal insufficiency) and also control group significant increasing of frequency of haplotype DR3-DQ2 (DBR1*17-DQA1*0501-DQB1*0201) was identified in patients from both groups with APS of adults in comparison with the control group. For the APS type 2, 4 against healthy control group - (p < 0.01); OR=6.4114, 95 % CI [3.0650 – 13.4114]; for the group of APS type 3 against healthy control group - (p < 0.05); OR=2.4286, 95 % CI [1.0733 – 5.4950] in accordance.

Increasing of frequency of haplotype DR3-DQ2 in patients with APS type 2, 4 in comparison with APS type 3 may indicate the association of this haplotype with the development of chronic adrenal insufficiency, independently from other autoimmune diseases (p < 0.02); OR=2.6400, 95 % CI [1.1975 – 5.8201].

Association of haplotype DR4-DQ8 (DBR1*04-DQA1*0301-DQB1*0201) was determined with the development both APS type 2, 4 (p<0.0001); OR=5.1985, 95 % CI [2.5322 –10.6724], and APS type 3 (p<0.0001); OR=6.3125, 95 % CI [3.1921 –12.4832] separately. There were no statistically significant differences in the prevalence of haplotype in the group of APS type 2, 3 and without chronic adrenal insufficiency.

According to the work of Huang and co-authors the association with haplotype HLA DR4- DQB1*0302 in patients with APS type 2 was traced only with type 1 Diabetes [7].

Presence of heterozygote genotype DR3-DQ2/DR4-DQ8 in patients with APS type 2, 4 considerably increases the risk of development of the disease in comparison with control group (p<0.0001); OR=38.2143, 95 % CI [8.1286 –179.6352] and with group of patients with APS type 3 (p<0.01); OR=4.2857, 95 % CI [1.4423 –12.7347]. It is an independent risk factor of development of APS type 2, 4.

Frequency of occurrence of heterozygote genotype DR3-DQ2/DR4-DQ8 in patients with APS type 3 is also higher in comparison with the control group (p<0.01); OR=8.9167, 95 % CI [1.7223 –46.1644].

This material matches the results of Myhre and co-writers, in which significant association of development of Addison’s disease was determined with the haplotypes DBR1*0404-DQA1*0301-DQB1*0302, and DBR1*0301-DQA1*0501-DQB1*0201, especially with the heterozygote genotype DR3-DQ2/DR4-DQ8 [8].

According to the data received from Albergoni and co-writers in which APS type 2 patients were included (n=54), and also materials by Erichsen and co-writers, in which there were patients with the primary chronic adrenal insufficiency (n=425), the association of both haplotypes DR3-DQ2 and DR4-DQ8 HLA II class was revealed with the development of primary chronic adrenal insufficiency independently from existence of type 1 Diabetes and autoimmune thyroid disease [2, 6].

Also, in foreign studies the effect of separate alleles on the development of APS was found. An increased occurrence of DQA1*0301 in APS type 2 and 3 was determined in comparison with isolated autoimmune diseases, which is an additional risk factor for the development of APS [9].

These data are confirmed by the results of this study, according to which the incidence of DQA1*0301 allele is significantly higher in patients with APS of adults compared to the control group (p <0.0001); OR= 4.2794, 95% CI [2.5006 – 7.3236], and the presence of the genotype DQA1*0301/DQA1*0501 also significantly increases the risk APS of adults in comparison with the control group (p <0.0001); OR = 13.8971, 95% CI [4.6163 – 41.8363].

Allele DQA1*0501 is significantly more present in patients with APS type 2, 4 compared to patients with APS type 3 (p <0.01); OR = 2.8947, 95% CI [1.4518 - 5.7717], as well as in APS type 2, 4 patients compared to the control group (p <0.01); OR = 2.5698, 95% CI [1.4733 -4.4823].

The genotype DQA1*0301/DQA1*0501 associated with the development of APS type 2 and 4 (p<0.0001); OR=21.00, 95% CI [6.3537 –69.4079] and APS type 3 (p<0.01); OR=9.3145, 95% CI [2.7706 –31.3141] and occur more often compared to the control group.

Besides predisposing genes to the development of chronic adrenal insufficiency, there are also protective. According to Betterle et al. DRI, DR7, DR13 and DR14 are such genes for APS type 2, frequency of which significantly higher in the control group in comparison with patients with APS type 2 (p < 0.05) [5].

Thus, in particular, there is a negative correlation of the development of autoimmune primary adrenal insufficiency with the presence of haplotypes DBR1*0101-DQA1*0101-DQB1*0501 (p < 0.0001) [8, 13], DBR1*0701-DQB1*0202 [14] and DBR1*13 (p < 0.02) [3, 4, 8].

In this study, among the protective HLA II alleles (Table 1), there was a significant increase of the frequency of occurrence alleles DBR1*07 (p < 0.02); OR = 0.4132, 95% CI [0.1962 - 0.8703]; DBR1*13 (p < 0.01); OR = 0.3062, 95% CI [0.1305 - 0.7184], as well as haplotype DBR1*07-DQB1*0201 (p < 0.05); OR = 0.4173, 95% CI [0.1830 - 0.9517] and DBR1*11-DQA1*0501-DQB1*0301 (p < 0.02); OR = 0.4105, 95% CI [0.118 - 0.8383] in the control group compared to the group of APS patients. These factors are inversely related to the probability of onset of APS of adults, which indicates their protective nature with respect to the development of the disease as a whole.

Significant differences in frequency of haplotypes DBR1*01-DQA1*0101-DQB1*0501 and DBR1*15-DQA1*0102-DQB1*0602/8, and also allele DBR1*01 in APS group and in the control group were not discovered.

DBR1*01 allele was determined as a protective one to the development of APS type 2 and 4 comparing with the group of APS type 3 and the control group (Table 2).

There was a significant decrease of the frequency of occurrence DRB1*01 allele in APS type 2 and 4 patients compared to the group of patients with APS type 3 (p < 0.01); OR = 0.0769, 95% CI [0.0098 -0.6038] and the control group (p <0.01); OR = 0.0996, 95% CI [0.0133 -0.7469]. Separately, no association was found for patients with APS type 3.

There was a decrease in the frequency of occurrence of the DRB1*13 allele among patients with APS type 2 and 4 in comparison with the control group (p < 0.01); OR = 0.0918, 95% CI [0.0123 -0.6865], which may indicate its protective properties with respect to the development of chronic adrenal insufficiency in APS of adults. However, significant statistical differences in the incidence of the DRB1*13 allele in the group of APS type 2 and type 4 and the group of APS type 3 were not found.

Haplotype DBR1*01-DQA1*0101-DQB1*0501 is associated with the absence of chronic adrenal insufficiency in APS. Frequency of this haplotype significantly lower in the group of APS type 2 and 4 in comparison with the group of patients with APS type 3 (p<0.01); OR=0.0769, 95 % CI [0.0098 –0.6038], and also in comparison with the control group (p<0.01); OR=0.1138, 95 % CI [0.0151 –0.8572].

Differences of frequency of this haplotype in patients with APS type 3 and in the control group are statistically unreliable.

The correlation with the allele DRB1*07, haplotype DRB1*07-DQB1*0201 and haplotype DRB1*11-DQA1*0501-DQB1*0301 as protective ones separately in patients with APS 2 and 4 types and, as well as in comparison with the APS type 3 group and the control group was not revealed.

Protective characteristics of DRB1*15-DQA1*0102-DQB1*0602/8 haplotype regarding the development of APS 2 and 4 types, and also APS 3 type were not discovered.
### Discussion

A strong association of haplotypes **DR3-DQ2, DR4-DQ8**, especially of genotype **DR3/DR4**, allele **DQA1*0301**, and also of genotype **DQA1*0301/DQA1*0501**, with the development of APS of adults was confirmed.

When the groups were divided into APS with the chronic adrenal insufficiency (APS 2 and 4 types) and APS without it (APS type 3), the strongest influence on the risk of the development of chronic adrenal insufficiency in APS had haplotype **DR3-DQ2 (DRB1 *17-DQA1 *0501-DQB1 *0201)**, as well as genotype **DR3 / DR4**. This fact can serve as an unfavorable prognostic sign for the development of chronic adrenal insufficiency in APS type 3 and require more thorough regular screening in such patients and their relatives with autoimmune diseases.

Herewith the presence of protective haplotype **DRB1*01-DQA1*0101-DQB1*0501**, and also allele **DRB1*01** and **DRB1*13**, in relation to development of chronic adrenal insufficiency in APS of adults oppositely allows to predict more favorable course of the syndrome.

Screening for the development of chronic adrenal insufficiency in APS type 3 patients is possible in standard terms - 1 time in 5 years.

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### Author contribution statement.

Troshina E.A. - formulation of the purpose and objectives of the study, development of the research concept, checking the text of the article

Larina A.A. - patient recruitment, static processing of received data, writing an article.

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