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

Trostina E.A., Larina A.A

Endocrinology Research Centre, Moscow, Russian Federation.

This research was conducted under the research grant RSF “Autoimmune endocrinopathies multiple organ failure that are genomic, postgenomic and metabolic markers. Genetic risk prediction, monitoring, early predictors, personalized correction and rehabilitation.” Project number is 17-75-30035.

Key words
Autoimmune polyglandular syndrome 2, 3, 4 types, chronic adrenal insufficiency, genes DRB1, DQA1, DQB1 HLA class II, protective haplotypes

Introduction
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
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. Haplotypes 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 risks 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 predisposal 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 set of QIAamp DNA Blood Mini Kit (QIAGEN). The method of multiprimer allele 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 and 95 % confidence interval (95 % CI). The strong association with the disease was considered significant with р<0,05. Association with gender was considered significant on gender (p=0,154).

Results

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

Comparing the two groups of patients with APS type 2, 3, 4 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 prevalence of vitiligo in adult APS patients was 11,5 %, alopecia - 5,1 %, celiac disease -- 2,56 %.

APS patients were separated into 2 groups: APS type 2 and 4 and with chronic adrenal insufficiency (N=36) and APS type 3 without chronic adrenal insufficiency (N=42). The two groups had significantly differences in the presence of type 1 Diabetes (33 % patients had type 1 diabetes in the group of APS type 2, 4 against 90% of APS type 3 (p < 0,01). Thyroid autoimmune diseases (AITD/Grave’s disease) existed in 89% of cases in the group of APS types 2, 4 and in 100 % - in the group of APS type 3 (according the classification of APS) (p < 0,05). The groups of patients with APS type 2, 4 and APS type 3 did not differ strongly according the frequency of occurrence of vitiligo – 6% against 17% in accordance (p =0,17) and alopecia 6% against 5% 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.

| t        | APS N=78 | Control group N=109 | APS vs. control group |
|----------|----------|----------------------|-----------------------|
|          | N        | %                    | n        | %                    | P OR; 95 % CI |
| DR3-DQ2  | 34       | 21,8                 | 14       | 6,4                  | p < 0,0001 OR 4.0609 |
|          |          | 95 % CI 2.0955 - 7.8695 |          |                      |               |
| DR4-DQ8  | 49       | 31,4                 | 16       | 7,3                  | p < 0,0001 OR 5.7815 |
|          |          | 95 % CI 3.1380 - 10.6520 |          |                      |               |
| DR3-DQ2/DR4-DQ8 | 21   | 26,9            | 2       | 1,8                  | p<0,0001 OR 19.7105 |
|          |          | 95 % CI 4.4618 - 87.0743 |          |                      |               |
| DRB1*01  | 14       | 8,9                  | 27       | 12,4                 | p=0,32        |
| DRB1*07  | 10       | 6,4                  | 31       | 14,2                 | p<0,02 OR 0.4132 |
|          |          | 95 % CI 0.1962 - 0.8703 |          |                      |               |
| DRB1*13  | 7        | 4,5                  | 29       | 13,3                 | p<0,01 OR 0.3062 |
|          |          | 95 % CI 0.1305 - 0.7184 |          |                      |               |
| DRB1*01-DQA1*0101-DQB1*0501 | 14 | 8,9 | 24 | 11 | p=0,60 |
| DRB1*07-DQB1*0201 | 8 | 5,1 | 25 | 11,5 | p<0,05 OR 0.4173 |
|          |          | 95 % CI 0.1830 - 0.9517 |          |                      |               |
| DRB1*11-DQA1*0501-DQB1*0301 | 11 | 7,1 | 34 | 15,6 | p<0,02 OR 0.4105 |
|          |          | 95 % CI 0.2011 - 0.8383 |          |                      |               |
| DRB1*15-DQA1*0102-DQB1*0602R | 7 | 4,5 | 6 | 2,6 | p=1,0 |
| DQA1*0301 | 54       | 34,6                 | 24       | 11                   | p<0,0001 OR 4.2794 |
|          |          | 95 % CI 2.5006 - 7.3236 |          |                      |               |
| DQA1*0501 | 52       | 33,3                 | 54       | 24,8                 | p=0,08        |
| DQA1*0301/DQA1*0501 | 27 | 34,6 | 4 | 3,7 | p<0,0001 OR 13.8971 |
|          |          | 95 % CI 4.6163 - 41.8363 |          |                      |               |

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 (DRB1*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 (DRB1*04-DQA1*0301-DQB1*0302) 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 APS group with chronic adrenal insufficiency or without chronic adrenal insufficiency.

According to the work of Huang and co-authors the association with haplotype HLA DR4-DQA1*0501/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 types 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 – 179.6532] 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 DRB1*0404-DQA1*0301-DQB1*0302, and DRB1*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].
### 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 *0101-DQA1 *0102-DQB1 *0501), 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.

Here with 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.

### Information about financing and conflict between interests.

The authors emphasize about absence of express or implied conflicts of interests, connected with conducting of this research and publication of this article.

### 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.

### References

1. Endokrinologiya: natsional'noe rukovodstvo / pod red. Dedova I.I., Mel'nichenko G.A.- M.: GEOTAR-Media, 2016 g. – 1081-1088 (In Russ.)
2. Albergoni P., Gazzola MV., Slanzi E., Carcassi C., Dal Pra C., Moscon A., Betterle C. HLA–DR and DQ associations with autoimmune Addison's disease in Italian patients Genes Immunity. 2003; 4(1): S33.

3. Baker P., Fain P., Kahles H., Yu L., Hutton J., Wenzlau J., Rewers M., Badenhoop K., Eisenbarth G. Genetic determinants of 21-hydroxylase autoantibodies amongst patients of the Type 1 Diabetes Genetics Consortium J Clin Endocrinol Metab. 2012; 97(8):E1573-8.

4. Betterle C., Lazzarotto F., Presotto F. Autoimmune polyglandular syndrome Type 2: the tip of an iceberg? Clin Exp Immunol. 2004; 137(2): 225–233.

5. Betterle C., Zanchetta R. Update on autoimmune polyendocrine syndromes (APS). Clinical Immunology and Allergology ACTA BIO MEDICA. 2003; 74: 9-33.

6. Erichsen M., Løvås K., Skinningsrud B., Wolff A., Undlien D., Svartberg J., Fougner K., Berg T., Bollerslev J., Mella B., et al. Clinical, Immunological, and Genetic Features of Autoimmune Primary Adrenal Insufficiency: Observations from a Norwegian Registry The Journal of Clinical Endocrinology & Metabolism. 2009; 94 (12): 4882-4890.

7. Huang W., Connor E, Dela Rosa T., Muir A., Schatz D., Silverstein J., Crockett S., She J-X., Maclaren N. Although DR3-DQB1*0201 may be associated with multiple component diseases of the Autoimmune Polyglandular Syndrome, the Human Leukocyte Antigen DRA-DQB1*0302 haplotype is implicated only in beta-cells autoimmunity J Clin Endocrinol Metab. 1996;81:2259-63.

8. Myhre AG., Undelien DA., Lovas K. et al. Autoimmune adrenocortical failure in Norway autoantibodies and human leukocyte antigen class II association related to clinical features J Clin Endocr Metab. 2002; 87:618–23.

9. Wallaschofski H., Meyer A., Tuschi U., Lohmann T. HLA-DQA1*0301-associated susceptibility for autoimmune polyglandular syndrome type II and III Horm Metab Res. 2003; 35(2):120-4.