Protective and predisposing genes to chronic adrenal insufficiency in patients with APS 2,3,4 types and Graves' disease - polymorphism of HLA II, CTLA-4 and PTPN22 genes

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Introduction: Autoimmune polyendocrine syndromes (APS) are a heterogeneous group of diseases characterized by the presence of autoimmune dysfunction of two or more endocrine glands and other non-endocrine organs.

The aim of the study was to determine the association of chronic adrenal insufficiency with polymorphism of *HLA II, CTLA-4* and *PTPN22* genes among patients with APS 2,3,4 types and patients with Graves' disease. The focus of the study was on the revealing of protective genes for Addison's disease in APS 3 type patients and in patients with Graves' disease.

Materials and methods: The case-control study involved 116 patients with APS 2, 3, 4 types, 95 patients with Graves' disease 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 was determined by the value of the odds ratio (OR - odd's ratio), the value of 95% confidence interval (95% CI).

Results: The frequency of *HLA II* class haplotype *DR*0301-DQA1*0201-DQB1*0501 (DR3-DQ2)* was increased in APS patients compared with the control group 21.8 % vs. 6.4% ($p < 0.001$); OR = 4.06, 95%CI [1.44-12.73].

The haplotype *DRB1*04-DQA1*0301-DQB1*0302 (DR4-DQ8)* showed the same results compared with the control group - 31.4 % vs. 7.3 % ($p < 0.001$); OR = 5.78, 95%CI [3.13-10.65]. The frequency of *DQA1*0301* allele in APS type 2, 3 ,4 group was 34.6 % vs. 11 % in the control group ($p < 0,001$) OR = 4.27, 95%CI [2.50-7.32]; genotype *DQA1*0301/DQA1*0501*- 34.6 % vs. 3.7 % respectively ($p < 0,001$) OR = 13.89, 95%CI [4.61- 41.83], and especially heterozygous genotype *DR3-DQ2/DR4-DQ8* - 26.9 % vs. 1.8 % ($p<0.001$); OR = 19.7, 95%CI [4.46-87.07].

There were no differences of occurrence the haplotype *DR3-DQ2* in the group of patients with Graves' disease- 27.4 % and APS patients – 21.8 % (p=0.3). The frequency of the haplotype *DR4-DQ8* was strongly lower in the Graves' disease patients – 9.8 % compared with APS of adults- 31.4% ($p < 0.001$); OR = 0.23, 95%CI [0.114-0.495] and didn't differ from the control group – 6.4% (p=0.51).

The frequency of heterozygous genotype *DR3-DQ2/DR4-DQ8* was also lower in Graves' disease group compared with APS patients group 2 % vs. 26.9 % ($p < 0.001$); OR = 0.065, 95%CI [0.015-0.29] and there were no statistical differences with the control group - 1.8% (p = 1.0).

The group of all APS patients was divided into two groups according to the manifestation of Addison's disease – APS type 2, 4 and APS type 3. The frequency of *DR3-DQ2* in patients with APS type 2, 4 was higher compared to APS type 3 – 30.5 % vs. 14.2 % ($p < 0.02$); OR=2.64, 95%CI [1.19–5.8]. There were no statistically significant differences in the prevalence of haplotype *DR4-DQ8* in the APS type 2, 4 group- 29.2% and APS type 3 -33.3 % (p=0.61).

The heterozygous genotype *DR3-DQ2/DR4-DQ8* found the strong association with the development of adrenal insufficiency in patients with APS types 2, 4 compared with the control group – 42 % vs. 1.8 % ($p<0.001$); OR=38.21, 95 %CI [8.12 –179.65], Graves' disease – 2 % ($p<0.001$); OR=29.64, 95 % CI [6.28-139.82] and the APS type 3 group – 14 % ($p<0.01$); OR=4.28, 95 %CI [1.44-12.73] separately.

Haplotype *DRB1*01-DQA1*0101-DQB1*0501* has been determined as protective for the development of Addison's disease. Frequency of *DRB1*01-DQA1*0101-DQB1*0501* was significantly lower in the group of APS type 2, 4 – 1.4 % comparing with the group of APS type 3 – 15.5 % ($p<0.01$; OR=0.0769), Graves' disease group – 12 % ($p <0.05$; OR=0.095) and the control group 11 % ($p<0.01$; OR=0.1138)

There were no significant differences in the frequency of occurrence of the haplotype - *DRB1*01-DQA1*0101-DQB1*0501* - in the type 3 APS groups, in patients with Graves' disease and in the control group (p=1).

A significant association between APS type 2, 3, 4 and the *CTLA-4 +49 A/G* genotype – 61.7 % comparing with Graves' disease group – 38 % was confirmed ($p < 0.01$); OR 2.63; 95%CI [1.29-5.32]. The frequency of *CTLA-4 +49 A/A* genotype was not significantly different in three groups (p=0.1).

PTPN22 +1858 T/C (rs 2476601) genotype significantly increases the risk of the onset of APS of adults 35.1 % compared with the control group – 12 % (p <0.05; OR 3.96; 95%CI [1.1-14.2]. The frequency of the *PTPN22 +1858 T/C (rs 2476601)* genotype was not significantly different in the Graves' disease group comparing with the control group and the APS type 2, 3 and 4 group.

Conclusion: *DR3-DQ2* haplotype predispose to APS of adults and Graves' disease separately. *DR3-DQ2* haplotype is an independent risk factor of the Addison's disease and development of APS type 2 in patients with APS type 3. *DR4-DQ8* haplotype in Graves' disease patients is a reliable risk factor of the development of APS of adults in such patients.

The heterozygous *DR3/DR4* genotype is the most predisposal risk factor of the onset of APS type 2 and 3 in Graves' disease patients and Addison's disease in APS type 3 patients.

*DRB1*01-DQA1*101*DQB1*501* haplotype has a significant protective effect on the development of APS type 2.

Gene polymorphism *CTLA-4+ 49A/G (rs231775)* and *PTPN22 +1858 T/C (rs2476601)* are predisposing to the development of APS 2, 3 types compared with the group of Graves' disease patients and the control group

The revealing of predisposing and protective genes to Addison's disease in patients with APS type 3 and Graves' disease will allow better predicting the risks of developing of the chronic adrenal insufficiency and the sudden onset of potential life-threatening complications (adrenal crisis) within the syndrome.