Table 1. Polymorphisms and methods used in the Gliwice study.
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Differences in genetic predisposition to Graves' disease (GD) and Graves' orbitopathy (GO) between young and elderly patients.'

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Introduction

Autoimmune thyroid disease (AITD) result from a combination of susceptibility alleles of many genes and environmental factors. In young patients, in whom the period of the environmental factors influence is shorter, the influence of the genetic predisposition to the development of complex diseases is more detectable. Association between genetic markers and age of diagnosis was reported in type I diabetes, rheumatoid arthritis and multiple sclerosis. Also the occurrence of Graves disease (GD) is more frequent in the children of young parents or families affected by the disease. Preliminary research of the whole genome analysis families suffering from GD confirmed association of FOXP3 gene with GD only with young sufferers, with age of onset ≤ 30 years of age. Also differences in the disease's phenotype between younger and older patients suggests different genetic predisposition to the GD. The question then arises whether and how differences in genetic predisposition are associated with GD and the occurrence of GO with the age of onset.

Aim of the study

The aim of the study was to evaluate the genetic predisposition to GD and GO (Graves orbitopathy) in young people. We analyzed polymorphisms of genes with proven relationship with GD, whose share in the predisposition to GD detected in reliance on the analysis of candidate genes: HLADRB1*03 gene HLADRB1, 1858T gene PTPN22, 49G gene CTLA4 and rs179247 and rs12101255 TSHR gene. Based on the positive results of the study of the entire genome the same criterion age of onset ≤ 30 years of age was applied.

Patients and methods

The analysis included 768 GD patients**.** Patients were divided into two groups using the criterion of age of onset: younger patients being diagnosed at ≤30 years of age (n = 226), and older patients with an age of onset >30 years of age (n = 542). Patients were consecutively recruited in the Department of Nuclear Medicine and Endocrine Oncology, Centre of Oncology in Gliwice, Poland (n=370) and in the Department of Endocrinology, Medical University of Warsaw, Poland (n=398).

Tabela 1. Clinical characteristics of patients with GD

	N=768
Gender (female : male)	617:151
Age of onset of GD in years (mean ± SD)	40.3 ± 4.49
GO present (NOSPECs≥2)	359 (46.7%)
Tobacco smokers	322 41.9%)
Disease duration in years: (mean ± SD)	2.72 ± 4.38

Tabela 2. Polymorphisms and methods used in the study.

Gene	Polymorphism (formerly) rs (HapMap)	Location	Method	PCR Primer F 5'-3' Primer R 5'-3'	PCR Annealing temperature	RFLP enzyme
HLA-DRB1	-	exon 2	PCR-SSP/ PCR-SSO	-	-	-
CTLA-4	A(49)G rs231775 / rs57563726	codon 1, exon 3	PCR-RFLP	F: CCAAGTCTCCACTTAGTTATCC R: CCTCCATCTTCATGCTCC	55,1°C	Bst71I, New England Biolabs
PTPN22	C(1858)T rs2476601	codon 620, exon 14	PCR-RFLP	F: TCACCAGCTTCCTCAACCACA R: GATAATGTTGCTTCAACGGAATTT	60°C	XcmI, New England Biolabs
TSHR	rs179247	intron 1	TaqMan SNP genotyping	-	-	-
TSHR	rs12101255	intron 1	TaqMan SNP genotyping	-	-	-

Statistical analysis

Statistical analysis was performed using the statistical program STATA12.0. The distribution of genotypes and alleles in both groups was compared using the chisquared test or the Fisher's Exact Test. Bonferroni correction was applied for multiple comparisons. Hardy-Weinberg equilibrium was analyzed. A multivariate logistic regression was used to determine the independent association between GD/GO, age of onset, genetic predisposition and smoking status..

Results

I. ASSOCIATION BETWEEN GENETIC PREDISPOSITION AND GD ONSET

UNIVARIATE LOGISTIC REGRESSION

Among the studied gene polymorphisms significant differences were found for HLA DRB1*03 polymorphism and 1858T polymorphism of PTPN22 gene, while comparing genotype frequency between patients with onset ≤ 30 years of age and olders (Figure 1).

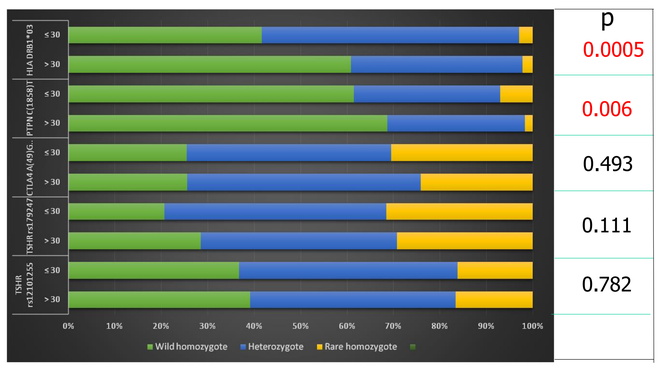


Fig.1. Differences in genotypes distribution in GD patients, non smokers, according to the age of onset

MULTIVARIATE LOGISTIC REGRESSION

Multivariate regression confirmed the results of the univariate regression (non-smokers) - in younger patients more common are polymorphisms of HLA-DR3 and PTPN22 genes compared with older patients (Figure 2).

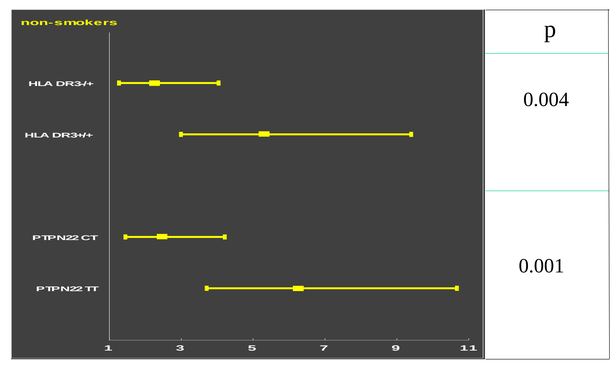


Fig. 2. Results of multiple linear regression analysis for GD patients with age of onset ≤ 30 years of age, non smokers

II. ASSOCIATION BETWEEN GENETIC PREDISPOSITION AND GRAVES' ORBITOPATHY

UNIVARIATE LOGISTIC REGRESSION ANALYSIS

In order to asses genetic predisposition to GO, we compared patterns of polymorphisms in the TSHR, PTPN22, CTLA4 and HLADRB1 genes in patients with and without orbitopathy. Results were analysed both for the group as a whole group, as well as for subgroups of younger (age at diagnosis 30 or less) and older (age at diagnosis greater than 30) patients. When the group was analyzed as a whole, as well as when older patients were analyzed alone, there was no difference found in allele frequency or distribution of genotypes for any of the analyzed polymorphisms. Analysis of the younger patient group revealed significant differences in the presence of polymorphism rs179247 of TSHR gene. The presence of a homozygous AA was associated with a significant reduction in risk of disease incidence, as compared to patients with AG or GG genotypes (p=0.019, OR=0.43).

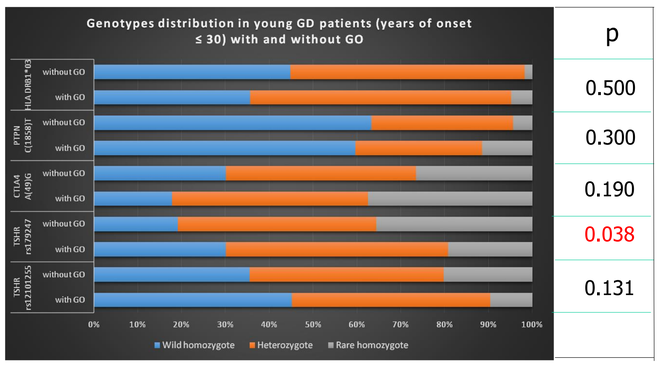


Fig.3. Differences in genotypes distribution in young GD patient with and without GO.

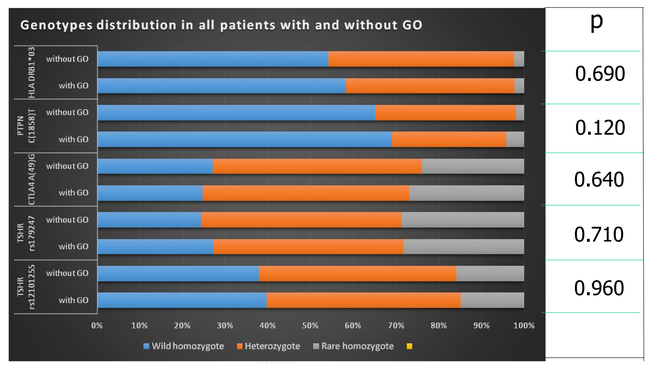
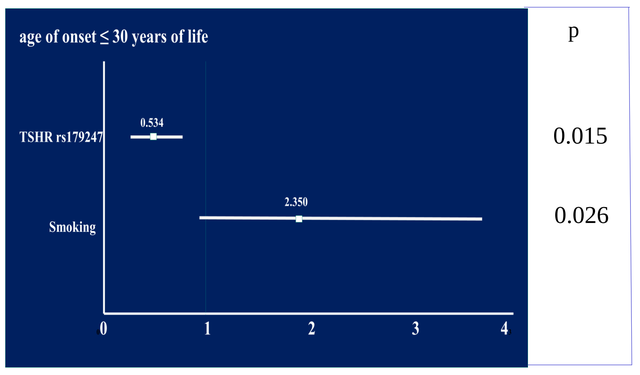
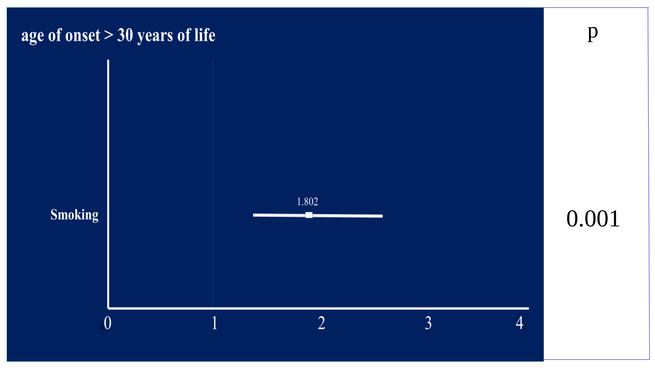


Fig.4. Differences in genotypes distribution in all GD patient with and without GO.

MULTIVARIATE LOGISTIC REGRESSION

Results of the logistic regression confirmed observations from analyses of alleles and genotypes. Genetic predisposition and smoking are independent risk factors with influences on development of ophthalmology in younger patients. The presence of the rs179247 polymorphism in the TSH receptor gene significantly lowers the risk of GO incidence (OR=0.534, p=0.015).





Conclusions

1. Polymorphism of HLADRB1*03 is associated with early age at diagnosis of Graves' disease.

2. Polymorphism rs179247 in the *TSHR* gene is associated with lower risk of graves orbithopathy in young patients with Graves disease.