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# The association between rs4684677 T/A polymorphism in preproghrelin gene and predisposition to autoimmune thyroid diseases in children.

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## BACKGROUND

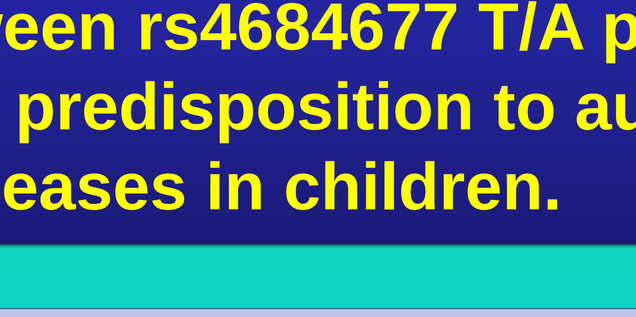
Ghrelin and obestatin are two gastrointestinal peptides obtained by post-translational processing of a common precursor, preproghrelin. Ghrelin is an orexigenic and adipogenic peptide and a potent growth hormone secretagogue (GHS) modified by the enzyme ghrelin-O-acyl-transferase to bind and activate its receptor, the GHS-R [1].Obestatin was initially identified as an anorexigenic peptide and as the cognate ligand for GPR39, but its effect on food intake and its ability to activate orphan G protein –coupled receptor GPR-39 (GPR39) are still controversial. The preproghrelin is a gene responsible for generation of ghrelin and obestatin [2,3]. mRNA expression for preproghrelin was found in AITDs in previous studies [4]. There are papers, where a role of preproghrelin polymorphism on various immunological diseases was determined, but nothing is known about its influence on the autoimmune thyroid diseases (AITDs). The aim of our study was to estimate the association of two polymorphism of preproghrelin gene with the predisposition to GD and HT in children.

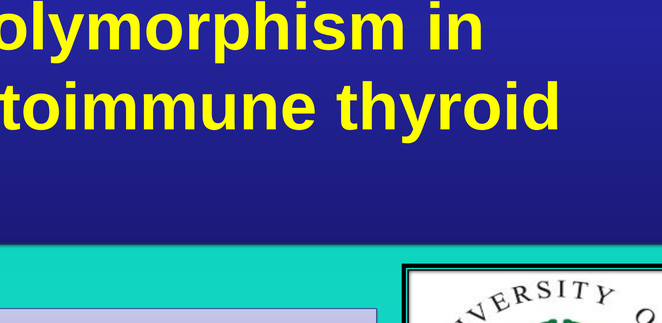
## DESCRIPTION OF METHODS

The study was performed in the group of 145 patients with GD (mean age, 16.5±2), 87 patients with HT (mean age, 15.2±2.2) and 161 healthy volunteers (mean age, 16.3±3). DNA was extracted from the peripheral blood leukocytes using a classical salting out method. The two SNPs rs696217 and rs4684677 in the ghrelin/obestatin prepropeptide gene were genotyped by TaqMan SNP genotyping assay (Applied Biosystems, USA). For all studied polymorphisms were used ready to use fluorogenic TaqMan assays: rs696217 (C_3151003_20) and rs4684677 (C_25607748_10). Reactions were carried out in a 7900HT Fast Real-Time PCR System (Applied Biosystems, USA) under the following conditions: 10 min at 95°C for starting AmpliTaq Gold activity, 40 cycles of 95°C for 15 s and 60°C for 1 min. As a negative control, we used a sample without template. The negative control was helpful for measuring any false positive signal caused by contamination. All SNPs were analyzed in duplicates. The levels of thyroid hormones, TSH and anti-thyroid autoantibody were determined using chemiluminescence method.

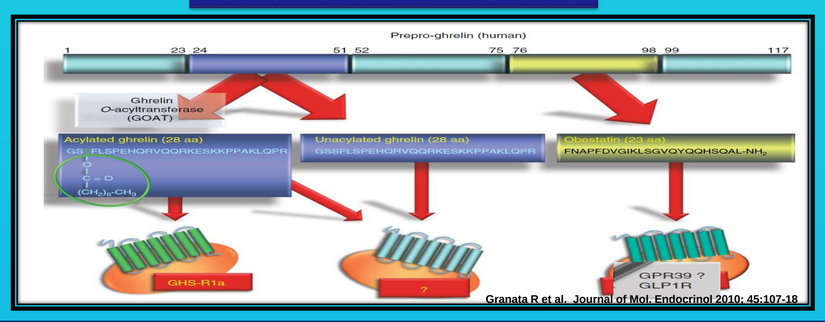
| | Graves' disease | Hashimoto thyroiditis | Controls | *p, **p |
|---|---|---|---|---|
| Female/male (n) | 145 (109/36) | 87 (74/13) | 161 (75/86) | |
| Age (years) | 16.5±2 | 15.2±2.2 | 16.3±3 | NS, NS |
| Weight(kg) | 55.19±2.39 | 58±5.28 | 60.9±7.8 | NS, NS |
| Height (cm) | 162.19±2.69 | 154.26±4.14 | 160±8 | NS, NS |
| fT4 (ng/dl) | 14.18±2.7 | 1.8±0.63 | 2.1±0.46 | *p<0.001, NS |
| fT3 (ng/dl) | 12.19±2.27 | 3.08±0.5 | 3.79±0.18 | *p<0.001, NS |
| TSH (µU/ml) | 2.37±2.1 | 9.87±4.37 | 3.04±0.72 | p<0.01, p<0.025 |
| TRAb (U/l) | 11.56±2.11 | 0.5±0.32 | 0.4±0.2 | *p<0.001, NS |
| anti-TGAb (IU/mL) | 347.49±86.7 | 620.98±240.34 | 91.6±30.46 | *p<0.01, **p<0.01 |
| anti-TPOAb (IU/mL) | 331.97±58.12 | 329.91±92.93 | 66±52.73 | *p<0.01, **p<0.01 |
| treatment | Methimazole | L-thyroxine | none | |

Clinical characteristics of patients with autoimmune thyroid disease and controls




REGULATORY ROLE OF GHRELIN AND OBESTATIN




## RESULTS AND CONCLUSION

In our study, rs4684677 T alleles was more frequent in patients with HT in comparison to healthy subjects (p=0.002) with OR = 8.0 and 95% confidence interval for OR: 1.8-206.7, what means that risk for development of HT is eight higher for T allel in comparison to A allel when considering CG as a point of reference. Also when considering only women group rs4684677 T alleles was more frequent in HT in comparison to healthy subjects (p=0.02) with OR = 6.7 and 95% confidence interval for OR: 1.2-168.37. Frequency of the SNP rs696217 is not different between the groups. There was significant relationship between rs4684677 polymorphisms and fT4 concentration (p=0.02) stated in women with HT and GD. When considering all patients (men and women with GD or HT) relationship between rs4684677 and fT4 concentration was observed (p=0.046). **In conclusion, the rs4684677 T/A polymorphisms in preproghrelin gene could contribute to autoimmue thyroid diseases development in children and T allel is the main risk factor.**

**rs696217**

| Group | Female | Male |
|---|---|---|
| GD | GG = 85 (85%)<br>TG = 15 (15%)<br>TT = 0 (0%)<br>G = 185 (93%)<br>T = 17 (7%) | GG=27 (79%)<br>TG=7 (21%)<br>TT = 0(0%)<br>G=61 (90%)<br>T=7 (10%) |
| HT | GG = 41 (84%)<br>TG = 8 (16%)<br>TT = 0 (0%)<br>G = 90 (92%)<br>T = 8 (8%) | GG = 9 (90%)<br>TG = 1 (10%)<br>TT = 0 (0%)<br>G = 19 (95%)<br>T =1 (5%) |
| C | GG = 67 (89%)<br>TG = 7 (10%)<br>TT = 1 (1%)<br>G = 141 (94%)<br>T = 9 (6%) | GG = 78 (91%)<br>TG = 8 (9%)<br>TT = 0 (0%)<br>G = 164 (95%)<br>T = 8 (5%) |

**rs4684677**

| Group | Female | Male |
|---|---|---|
| GD | AA = 0 (0%)<br>AT = 12 (12%)<br>TT = 88 (88%)<br>A = 12 (6%)<br>T = 188 (94%) | AA = 0 (0%)<br>AT = 2 (6%)<br>TT = 32 (94%)<br>A = 2 (3%)<br>T = 66 (97%) |
| HT | AA = 0 (0%)<br>AT = 1 (2%)<br>TT = 48 (98%)<br>A=1 (1%)<br>T = 97 (99%)<br>p<0.02 | AA = 0 (0%)<br>AT = 0 (0%)<br>TT = 10 (100%)<br>A=0 (0%)<br>T =20 (100%)<br>p<0.002 |
| C | AA = 2 (3%)<br>AT = 7 (9%)<br>TT = 66 (88%)<br>A=11 (7%)<br>T = 139 (93%) | AA = 3 (4%)<br>AT = 8 (9%)<br>TT = 75 (87%)<br>A=14 (8%)<br>T = 159 (92%) |

Fig. Distribution of genotypes and alleles of rs696217 and rs4684677 preproghrelin gene in groups with HT, GD and in control group. We used c2 tests and Fisher's exact test to evaluate the significance of differences in frequencies of genotype and allele among the subject groups.

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