Glucocorticoid receptor and HSD11B1 gene polymorphisms influence the therapy and therapy-associated morbidities in patients with Addison's disease

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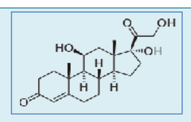
Background:



Glucocorticoids are steroid hormones responsible for a specific answer to stress. They exert their effects through the **glucocorticoid receptor (GR)** which is located - without ligand - in the cytoplasm in a multi-protein complex. Upon ligand binding conformation changing and translocation to the nucleus occurs. Homodimers of the GR bind to DNA through a specific DNA sequences (GRE-glucocorticoid response element) and stimulation or inhibition of the transcription of target genes containing GRE may occur. **The gene of the glucocorticoid receptor** map to 5q31, it contains 9 exons. More than 1000 ***GR* gene polymorphisms** were identified, the majority is located in intron sections . The most commonly studied SNPs of GR are ***N363S***, ***BclI.*** and ***A3669G***. Correlations were demonstrated between polymorphisms and decreased or increased sensitivity to glucocorticoids. Some polymorphisms may affect the dose of the glucocorticoid replacement. The local, cell-type specific glucocorticoid effect is modulated by the function of the **11-ß-hydroxysteroid dehydrogenase enzymes (11HSD)** responsible for the interconversion of cortisone and cortisol. The isoform 1 (11HSD1) is a NADP(H) dependent bidirectional enzyme, which mainly converts inactive cortisone to active cortisol. The isoform 2 is responsible for the opposit reaction. Individual sensitivity against glucocorticoids and activity of the HSD11B1 enzymes are at least partly determined by genetic factors, such as **polymorphisms of the HSD11B1 gene**, which map to 1q32-41 and contains 6 exons. The ***rs4844880*** SNP is located in the promoter region, and ***rs12086634*** in the 3. intron. For both SNPs various associations with clinical and laboratory parameters have been demonstrated.

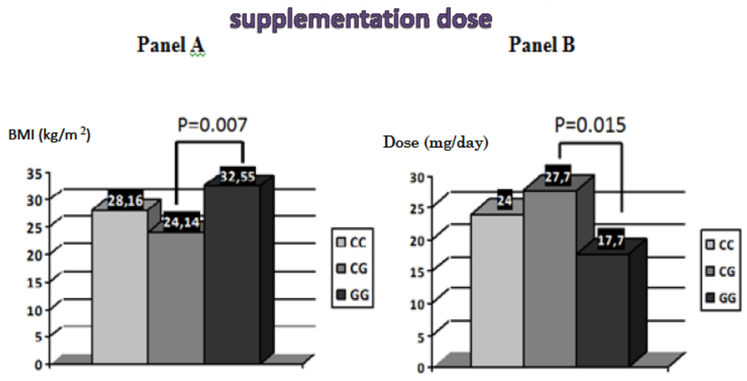
Aim:

- To examine the prevalence of three, well-characterized (N363S, BclI, 9ß or A3669G SNP (rs6198)) glucocorticoid receptor and two (rs12086634, rs4844880) HSD11B1 gene polymorphisms patients with Addison's disease
- To examine the relationship between the carries-state and the glucocorticoid replacement dosage, and clinical / laboratory parameters

Patients and Methods:

- Retro-and prospective study
- Patients with Addison's disease, primary adrenal cortical insufficiency (n=67), diagnosed and followed at the 2nd Department of Medicine, Semmelweis University
- Clinical, laboratory data and the dosage of the hormone replacement therapy were collected and analysed
- Population control for genetic study was formed from clinically healthy individuals (n = 160)
- Molecular biology methods
  - DNA isolation from peripheral blood samples
  - GR polymorphisms:BclI., N363S genotyped by allele-specific PCR & electrophoresis
  - A3669G, rs12086634, rs4844880 – by Taqman assay, Real Time PCR
- Statistical methods
  - Chi-square, Fisher test (distribution of allele frequencies)
  - ANOVA (mean values of continuous variables)

Assotiation between BclI. Polymorphism of the GR gene and the BMI andtotal daily hydrocortisone equivalent supplementation dose



Results:

- The allele frequency of ***N363S*** polymorphism was higher in patients compared to the control group (8.5% vs. 3.1%; p=0,019).
- The homozygous carriers of the ***BclI.*** had significantly higher BMI compared to the heterozygous carriers (p=0.007, power:100%), and the need the total hydrocortisone equivalent supplementation dose was significantly lower than in heterozygous carriers or non-carriers (p=0.015)
- The disease appeared earlier in ***A3669G*** polymorphism carriers (34.5 vs. 42.3 years, p=0.011).
- The BMI of the carriers of the ***rs4844880*** carriers was significantly higher compaired to non-carriers (31.46 vs. 24.46 kg/m2, p=0.003), and the body weight adjusted supplementation dose was significantly lower (0.284 vs. 0.409 mg/kg/day, p=0.031, power:87.5%).
- ***rs12086634*** polymorphism showed an association with the annual change in the Z-score measured at the lumbal spine. In carriers a negative tendency while in non-carriers a positive tendency was observed (-0.065 vs. 0.115, p=0.01).
- Annual decrease in bone mineral density, T-score and Z-score at lumbal spine were significantly lower in ***rs4844880*** carriers compairing to the non-carriers (p=0.017, 0.005 and 0.003, power: 92.1%, 90.7%, 96.2%)
- The effect of the ***rs4844880*** polymorphism on BMI and bone scores is keeped in case of dexamethason non-takers, but none of these associations was detected in dexamethasone treated patients.
- Females with the age over 50 years, the ***rs4844880*** polymorphism had a benefitial effect on the bone values while In female patients between 0-50 years, associated with body weight and BMI

P values of statistical analysis used for evaluation of the relationship between polymorphisms and laboratory parameters and therapeutic dose of patients with Addison disease

	BclI.	N363S	A3669G	rs12086634	rs4844880
BMI	0.018*	0.11	0.82	0.397	0.016*
Age of the apperance of disease	0.29	0.69	0.011*	0.92	0.093
Body weight change	0.24	0.32	0.99	0.38	0.25
Total hydrocortisone equivalent supplementation dose	**0.015**	0.73	0.63	0.26	0.11
Body weight adjusted supplementation dose	**0.011**	0.55	0.53	0.14	0.031*
RBC	0.89	0.55	0.8	0.56	0.12
Hb	0.2	0.96	0.99	0.27	0.074
Htc	0.42	0.93	0.97	0.14	0.084
Fasting blood glucose	0.8	0.92	0.59	0.86	0.6
Se. cholesterine	0.94	0.62	0.096	0.22	0.59
Se. Triglyceride	0.066	0.11	0.66	0.28	0.21
Se. LDL cholesterine	0.76	0.4	0.27	**0.037**	0.25
Density at FN	**0.01**	0.89	0.95	**0.012**	0.92
Annual change in density at FN	0.369	0.65	0.95	0.414	0.6
Density at LS	**0.048**	0.42	0.11	0.104	0.31
Annual change in density at LS	0.121	0.71	**0.019**	0.051	0.017*
T-score at FN	**0.002**	0.97	0.82	**0.002**	0.82
Annual change in T-score at FN	0.824	0.98	0.79	0.28	0.66
T-score at LS	**0.038**	0.2	0.13	**0.017**	0.32
Annual change in T-score at LS	0.441	0.86	0.067	0.137	0.005*
Z-score at FN	**0.001**	0.8	0.52	**0.001**	0.78
Annual change in Z-score at FN	0.461	0.71	0.78	0.245	0.65
Z-score at LS	**0.013**	0.44	0.11	**0.026**	0.19
Annual change in Z-score at LS	0.582	0.87	**0.019**	0.01*	0.003*

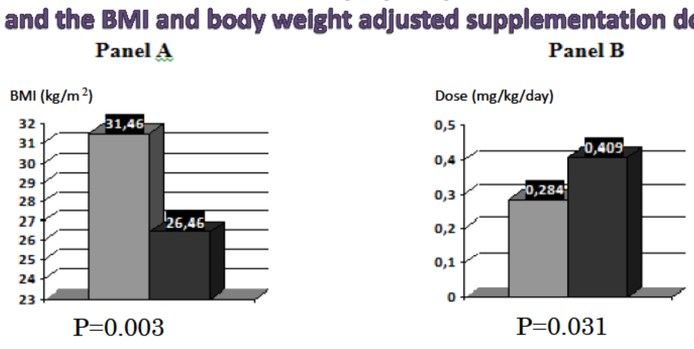
BMI-body mass index (kg/m²), RBC-red blood cell, Hb-hemoglibine, Ht-hematocrite, LDL-low density lipoproteine, FM-femoral neck, LS-lumbal spine, * p<0.05, power>80%; values in bold showed reached significancy but with insufficient power.

P values of ANOVA test used for evaluation of the relationship between HSD11B1 polymorphisms and BMI and bone parameters depending on wether taking Dexamethasone

	rs12086634		rs4844880	
	Dexamethasone treated group	No dexamethasone	Dexamethasone treated group	No dexamethasone
BMI	0.125	0.232	0.698	0.002*
Density at FN	0.704	0.520	0.121	0.888
Annual change in density at FN	0.259	0.906	0.757	0.568
Density at LS	0.817	**0.036**	0.495	0.290
Annual change in density at LS	0.163	0.191	0.955	0.013*
T-score at FN	0.586	0.340	0.118	0.797
Annual change in T-score at FN	0.121	0.832	0.948	0.394
T-score at LS	0.868	0.003*	0.770	0.307
Annual change in T-score at LS	0.294	0.632	0.188	0.003*
Z-score at FN	0.681	0.417	0.465	0.669
Annual change in Z-score at FN	0.294	0.642	0.668	0.623
Z-score at LS	0.995	**0.011**	0.627	0.184
Annual change in Z-score at LS	0.093	0.077	0.226	0.002*

BMI-body mass index (kg/m²), FM-femoral neck, LS-lumbal spine, * p<0.05, power>80%; values in bold showed reached significancy but with insufficient power.

Association between rs4844880 polymorphism of the HSD11B1 gene and the BMI and body weight adjusted supplementation dose



P values of ANOVA test used for evaluation of the relationship between *rs4844880* polymorphism and BMI, hydrocortisone supplementation dose and bone parameters depending on the age in case of female patients with Addison's disease

	Age: 0-50 years	Age over 50 years
Body weight	0.023	0.338
BMI	0.005*	0.476
Annual change in body weight	0.035*	0.154
Triglyceride	**0.028**	0.249
Body weight adjusted supplementation dose	0.231	**0.029**
Annual change in density at LS	0.442	0.0001*
Annual change in T-score at LS	0.709	0.0001*
Annual change in Z-score at LS	0.463	0.0001*

BMI-body mass index, FN-femoral neck, LS-lumbal spine, * p<0.05, power>80%, values in bold showed reached significancy but with insufficient power.

Allele frequencies of the investigated SNPs in patients and controls

	Patients with Addison's disease (n=67)		Healthy controls (n=160)	
	Number	Allele frequency	Number	Allele frequency
BclI.		25.4%		34%
CC	37		54	
CG	26		61	
GG	4		14	
N363S		8.5%*		3.1%
AA	54		122	
AG	11		7	
GG	0		0	
A3669G		19.4%		22%
AA	41		82	
AG	26		37	
GG	0		10	
Rs12086634		14,18%		17.5%
GG	49			
GT	17			
TT	1			
Rs4844880		14,93%		18.2%
TT	49		167	
TA	16		75	
AA	2		8	

Conclusion:

- In Hungarian patients with Addison's disease the allele frequency of ***N363S*** polymorphism was higher than in the control group .
- The disease manifests earlier in carriers of ***A3669G*** polymorphism, which may refer to the predisposing role of this SNP for adrenocortical insuficiency
- The ***Bcl1*** polymorphism in homozygous form significantly affects the dose requirement of the hormone replacement therapy, the higher BMI and lower need of supplementation dose may confirm the sensitizing effect of this SNP against glucocorticoids.
- Carriers of the ***rs4844880*** polymorphism had favorable changes in bone density compaired to the non-carriers, but this effect wa most pronounced in postmenopausal women.
- In premenopausal women, ***rs4844880*** polymorphism associated with body weight.
- In Dexamethasone treated patients correlations observed in case of HSD11B1 polymorphisms were not presented, suggesting that the 11-ß-hydroxysteroid dehydrogenase enzyme is involved in the metabolism of exogenous administered hydrocortisone as well.

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