Genetic factors influencing glucocorticoid sensitivity

Ph.D. theses

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I. Introduction

Glucocorticoids are essential steroid hormones involved in the regulation of many physiological processes including adaptation to stress, carbohydrate-, protein-, fat-, calcium- and bone- metabolism, immune function, growth and behavior regulation. They play role in the therapy of several diseases, such as autoimmune diseases, severe infections, nephrotic syndrome, certain dermatological, allergic, respiratory, haematological, neurological disorders and in inhibition of rejection following transplantation. Lack of glucocorticoids, for example in Addison's disease or hypopituitarism, patients need lifelong hormone substitution therapy. The accurate adjustment of the therapy is indispensable to minimize the appearance of the side effects. In healthy individuals, the serum cortisol levels shows diurnal rhythm, which is not reproducible with the current available therapeutic opportunities. This rhythm is influenced by age, gender, cortisol binding globulin (CBG) level, body mass, individual glucocorticoid sensitivity and metabolism.

Glucocorticoids exert their action through the intracellular glucocorticoid receptor (GR). The gene of the GR is located on the long arm of chromosome 5. Currently, dbSNP (dbSNP - National Center for Biotechnology Information; www.ncbi.nlm.nih.gov/SNP) lists more than 3000 active SNPs in the human GR gene. The most investigated SNPs are N363S, BclI:, ER22/23EK and A3669G, since in case of these polymorphisms, associations with increased or decreased sensitivity to glucocorticoids have been reported.

The locally available glucocorticoid level is modulated by the 11ß-hydroxysteroid dehydrogenase (11ß-HSD) enzymes. The 11β-HSD2 converts the biologically active cortisol to the inactive cortisone, protecting the mineralocorticoid receptor from the activation by glucocorticoid excess. The 11β-HSD1 is a NADPH-dependent, bidirectional enzyme with both dehydrogenase and reductase effects. Reductase function converts the biologically inactive cortisone to the active cortisol, thereby it enhances the activation of the GR. The different regulation of the enzyme in different tissues may play role in the pathomechanism of some diseases, such as obesity, metabolic syndrome, polycystic ovary syndrome, rheumatoid arthritis and osteoporosis. Single nucleotide polymorphisms (SNPs) of the HSD11B1 gene influence the activity of the enzyme, thereby the accessible hormone quantity to the GR. As a result, the tissue-specific glucocorticoid sensitivity may be modified at prereceptorial levels. Variants of the HSD11B1 gene modulate the enzyme activity hence the availability of cortisol.

The prevalence of primary adrenal deficiency, also known as Addison’s disease is 140/1.000.000 inhabitant. Nowadays, the primary pathogenetic factor is the autoimmune adrenalitis. The main symptoms of hypadraenia are the progressive weakness, fatigue, hypotension and collapse, nausea, and weight loss, either anorexia and hyperpigmentation on pressure-exposed body surfaces in case of long-term, untreated disease. Sometimes the symptoms of Addison's crisis are the first to call attention to the disease. The treatment of Addison's disease is based on the replacement of mineralocorticoid and glucocorticoid hormones. Both the disease and the life-long glucocorticoid replacement therapy have a negative impact on the quality of life of the patients.

Glucocorticoid resistance, also named Chrousos syndrome, is a rare, sporadic or familial condition characterized by symptoms developed due to local, tissue-specific or generalized symptoms due to partial insensitivity to glucocorticoids. Due to this insensitivity, and thereby inadequate negative feedback, ACTH, and consequently cortisol production are compensatory stimulated. The disease is characterized by biochemically proved hypercortisolism without the clinical stigmata of Cushing syndrome. The chronic excess of ACTH results in an overstimulated steroid biosynthesis, including increased production of adrenal steroids with androgenic and/or mineralocorticoid activity. The clinical spectrum ranges from a completely asymptomatic form to severe, life threatening conditions. The molecular basis of the disease was attributed to mutations in the glucocorticoid receptor gene. To date more than 15 different mutations of the GR that cause glucocorticoid resistance have been identified.

II. Objectives

I studied the effect of the genetic variants of the glucocorticoid receptor and the 11-ß-hydroxysteroid dehydrogenase enzyme on the hormone replacement therapy and the appearance of the therapy-associated side effects in the group of patients with Addison’s disease followed at the 2nd Department of Medicine, Semmelweis Univrsity. My aims were:

1. To investigate the allele frequency of the most commonly studied polymorphisms of the glucocorticoid receptor gene (BclI., N363S and A3669G), and the HSD11B1 gene (rs12086634 and rs4844880, which was first described by our team), and to compare these results with the international published data.
2. The description of the correlations between the carrier state of the investigated polymorphisms and the clinical parameters, in order to study the associations of SNPs on the appearance of side effects in hormone replacement therapy.
3. To examine the relationship between the carrier state of the SNPs and the temporal appearance of the disease, in order to detect the possible predisposing factors to Addison’s disease.
4. To explore the effect of the polymorphisms on the need of hormone replacement dosage, in order to determine an additional criteria for optimalization of the individualized therapy.
5. To asses the effect of HSD11B1 polymorphisms on bone metabolism.
6. To separately investigate the effects of the HSD11B1 polymorphisms on the metabolic parameters in patients with Addison’s disease taking or not taking Dexamethasone, taking into account that the 2nd type of 11-ß-HSD enzyme does not oxidize Dexamethasone but hydrocortisone.
7. To separately investigate the effects of the rs4844880 polymorphism on bone metabolism in pre- and postmenopausal women, in order to detect, whether hormonal changes caused by menopause influence the effect of this SNP on the clinical parameters.
8. To identify pathogenic GR mutation in a 31-year-old woman with Chrousos syndrome diagnosed and treated at the 2nd Department of Medicine, Semmelweis University, and to summarize the mutation-associated genotype-phenotype correlations.

III. Methods

III.1. Subjects and controls

Patients with Addison's disease (n=68) diagnosed and followed up at the 2nd Department of Medicine, Semmelweis University, Budapest were enrolled. In these patients, the adrenal insufficiency was related to autoimmune adrenalitis, patients receiving glucocorticoid hormone replacement for other reasons (s.a. congenital adrenal hyperplasia, adrenal tuberculosis, sarcoidosis, amyloidosis, haemochromatosis, malignant or hematological diseases, adrenal haemorrhage) were excluded, moreover, isolated ACTH deficiency, or other pituitary disorders did not appear on the basis of laboratory data. The diagnosis of primary adrenal insufficiency was based on clinical signs, electrolyte abnormalities (hyponatraemia, hyperkalaemia), strengthened by further laboratory tests: low serum cortisol levels, which did not show a sufficient increase due to stimulation after administration of Synacten (after administration of intravenous ACTH1-24, after 60 minutes, the serum cortisol level does not rise above 20 ug/dl). The plasma ACTH level measured at the diagnosis was high in all patients (1381±1523 pg/ml) whom this data was available (in 56 of 68 patient). 21-hydroxylase antibody levels at the diagnosis were available only in 5 cases, with strong positivity. Patients receiving further steroid therapy for other reasons (s.a. rheumatoid arthritis) or taking medications that affect the metabolism of hydrocortisone were excluded. For the genetic association study we used as a population control a group of clinically healthy individuals (n = 160 in case of GR polymorphisms and n=250 for the HSD11B1 polymorphisms). In these individuals no sign of any endocrine disease has been observed.

III.2. Clinical and laboratory parameters

Anthropometric parameters, i.e. age, height (cm), body weight (kg) and body mass index (BMI) were collected. In addition, age at the beginning of hormone substitution, changes in body weight during therapy (kg / year), glucocorticoid hormone replacement dose expressed as hydrocortisone equivalent (mg / day), and adjusted for body weight (mg / kg / day) were also noted. Other medicines affecting the metabolic status (antihypertensive, cholesterol-lowering, antidiabetic therapy) and hormone preparations (thyroid, growth and sex hormones) were also recorded.

Laboratory measurements were performed at the Central Laboratory of Semmelweis University. From each patient fasting blood samples were obtained between 08:00 and 09:00 h. Laboratory measurements included complete blood cell count, hepatic and kidney functions,

glucose and detailed lipoprotein measurements. Serum, salivary and urinary cortisol, plasma ACTH, serum estradiol, progesterone, sex hormone binding globulin (SHBG), testosterone, luteotropic hormone (LH), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), free thyroxine (fT4), prolactine and growth hormone (GH) concentrations were measured with electrochemiluminescence immunoassays, and serum dehydroepiandrosterone sulphate (DHEAS) and androstendion concentrations with radioimmunoassay .

III.3. Bone mineral density measurement

The bone mineral density was measured using DEXA (QDR 4500C, Hologic Inc., Waltham, MA, USA; Software version 9.03) in the regions of femoral neck and lumbal spine (L1-4). The surface density of the bones was determined (bone mineral density, BMD, g/cm2), and T-score values (in spreading unit) for the regions were calculated according to the deviation from the peak-bone density of the same-sex young population. The deviation from the age and gender specific averages (Z-score) was also calculated. These data were compared to the previous results of patients on the same instrument, and annual BMD, T- and Z-score changes were calculated. The reference values given by the manufacturer were used for the measurements.

III.4. Molecular Biological Methods

III.4.1. DNA extraction

DNA was isolated from peripheral blood samples by standard procedures using commercially available DNA isolation reagents (DNA Isolation from mammalian blood, ROCHE Gmb, Basel, Swizterland and DNA Isolation kit from blood, Qiagen, San Diego, USA) kits. The DNA samples were stored at -80 ° C until use.

III.4.2. Allele-specific PCR

Allele-specific PCR reaction was used to the investigation of the BclI. and N363S polymorphisms. In case of BclI. polymorphism, I used the following primers for the amplification: forward (F): 5’-GAGAAATTCACCCCTACCAAC-3’; reverse (R): 5’-AGAGCCCTATTCAAACTG-3’; wild forvard (VF): 5’-CAATTCCTCTCTTAAAGAGATT-3’; mutant reverse (MR): 5’-GACAAGTTATGTCTGCTGATG-3’ (Invitrogene Life Technologies, Glasgow, UK).

The PCR reaction was carried out using ThermoCycler (ProFlex PCR System, ThermoFisher Scientific, USA). The PCR mixture with the final volume 25 μl contained 0.3 μmol/l of each of the listed oligonucleotide primers,10 mmol/l Tris–HCl, 2.5 mmol/l MgCl2, 50 mmol/l KCl, 0.2 mmol/l deoxynucleotide triphosphate, 0.5U Taq polymerase (2×ImmoMix, Bioline, UK) and 5% glycerol. The program starts with a 7-minute denaturation at 95˚C followed by a 3-stage, 38-cycle phase: 95˚C, 56˚C and 72˚C for 45-45 seconds, and finally, it is closed by a 10 minute extension at 72 ° C.

For N363S polymorphism, I used the following primers for the amplification:
2/4 F: 5'-CCAGTAATGTAACACTGCCCC-3'; 2/4 R: 5'-TTCGACCAGGGAAGTTCAGA-3'; 363-MR: 5'-ATCCCTGGCACCTATTCCAAC-3'
(Invitrogene Life Technologies, Glasgow, UK). The reaction was carried out according to the protocol elaborated previously by our team. The program starts with a 5-minute denaturation at 95˚C followed by a 3-stage, 34-cycle phase: 95˚C, 61˚C and 72˚C for 1-1 minutes, and finally, it is closed by a 10 minute extension at 72 ° C.

The amplified DNA was separated by gel electrophoresis on 2% agarose gel in case of BclI. and 3% agarose gel in case of N363S polymorphism, and visualized with ethidium bromide under ultraviolet transilluminator.

III.4.3. Real time PCR

The A3669G, rs12086634 and rs4844880 (HSD11B1) polymorphisms were detected using a primer-probe set purchased as predesigned Taqman allelic discrimination assay. The assay was performed according to the manufacturer's instructions (Applied Biosystems, Life Technologies, Carlsbad, California, USA) on a 7500 Fast Real Time PCR System (Applied Biosystems). The procedure is based on the fact that two different fluorophor dye-labeled (FAM and VIC in this case) probes can be ligated to the wild and mutant nucleotide sequences at the site of the SNPs which are included in the reaction mixture prepared by the manufacturer. In case of carrying or non-carrying the SNP, during the reaction, one of the two probes binds, the fluorophore dye is released from the inhibition, and emit fluorescent signal, which is proportional to the amount of PCR product, and it can be measured and visualized on graph. In case of homozygous wild type, only one (in this case FAM) curve can be observed, in case of homozygous mutant type, the other dye (VIC) gives the signal, while in case of heterozygous carrier status, signal intensity of both dyes can be detected. The parameters of the program were the followings: 2 minutes at 50 ° C followed by a 10-minute denaturation at 95 ° C, followed by a 2-stage, 40-cyclic phase: 95 ° C for 15 seconds and 60 ° C for 1 minute.

III.4.4. Sanger sequencing of the GR gene

The nucleotide sequence of the glucocorticoid receptor coding region was determined by direct DNA Sanger sequencing, in order to identify the possible pathogenic mutation. The whole coding region of the GR was amplified by polymerase chain reaction using original forward and reverse oligonucleotide primers described by Koper et al. 100 ng of DNA samples were amplified in a ThermoCycler (ProFlex PCR System, ThermoFisher Scientific, USA) in a volume of 50µl, which contains 0.3 µmol/l of each oligonucleotide primer (Invitrogene Life Technologies, Glasgow, UK), 10mmol/l Tris–HCl, 2.5 mmol/l MgCl2, 50 mmol/l KCl, 0.2 mmol/l deoxynucleotide triphosphate, 0.5U Taq polymerase (Pharmacia Biotech, Uppsala, Sweden) and 5% glycerol. The PCR protocol was started with a denaturation (10 min, 95˚C), followed by 35 cycles of 45 sec each at 95, 60 and 72 ˚C, and finished with an extension at 72 ˚C for 10 min. PCR products were purified on 1% agarose gel with DNA/RNA extraction kit (Viogene, New Taipei City, Taiwan). PCR-amplified DNA was sequenced using BigDye Terminator Cycle-Sequencing Kit (Applied Biosystems, Foster City, CA) direct cycle sequencing, and run on an automated sequencer 310 Genetic Analyser from Applied Biosystems. Final products were purified with gel-filtration (NucleoSEQ, Macherey-Nagel, Düren, Germany).

III.5. Statistical analysis

Distribution of allele frequencies was analyzed using the Chi-square, Fisher's exact test. ANOVA was used for the analysis of the relationship between GR and HSD11B1 polymorphisms and the mean values of continuous variables (IBM SPSS Statistics version 19.0, IBM Corp., Armonk, New York, USA). Power calculation was performed using a freely available algorithm (https://www.dssresearch.com/Knowledge Center/toolkitcalculators/statisticalpowercalculators.aspx). A values are presented as mean ± SD. P < 0,05 and power >80% were considered to be significant.

III.6. Three-dimensional protein modeling

To investigate the effect of the mutation identified in the GR gene on the protein structure, molecular modeling and analyses were performed using the UCSF Chimera package. The coordinates of the GR ligand binding domain have been obtained from PDB structure 4UDC.

IV. Results

IV.1. Allele frequencies and associations of the GR polymorphisms with phenotype features

IV.1.1. BclI. Polymorphism of the *GR*

The allele frequency of the BclI. polymorphism located in the 2nd intronic region of the GR did not differ between patients and controls. The SNP showed a significant association with the body mass index (BMI, kg/m^2). Homozygous carriers had significantly higher BMI compared to heterozygous carriers (p=0.007; power 100%), and the total hydrocortisone equivalent substitution dose was significantly (p=0.002, power: 98.9%) lower in these patients than in heterozygous or wild-type carriers.

IV.1.2. N363S polymorphism of the *GR*

The allele frequency of the N363S polymorphism was higher in patients compared to the control group (8.0% vs. 3.1%; p=0,019).

IV.1.3. A3669G polymorphism of the *GR*

The allele frequency of the A3669G polymorphism did not differ between patients and controls. It is particularly interesting that the disease appeared significantly earlier in carriers of the A3669G polymorphism compared to non-carriers (34.5 ± 12.2 vs. 41.05 ± 12.35 years, p=0.04). Moreover, carriers had significantly higher serum ACTH levels at the diagnosis of the disease (1944±478 vs. 984±144 pg/ml; p=0.023, power: 100%).

IV.2. Allele frequencies and associations of the HSD11B1 polymorphisms with phenotype features

IV.2.1. rs12086634 polymorphism

The allele frequency of the rs12086634 polymorphism did not differ between patients and controls. The SNP showed a significant association with the annual change of the Z-score at the lumbal spine region: the annual decrease in the Z-score levels was significantly higher in carriers compaired to the non-carriers (-0.12±0.18 vs. -0.07±0.13, p=0.01). In addition, significantly lower bone mineral density, T- and Z-scores measured at the femoral neck region, while significantly higher T- and Z-scores measured at the lumbal spine region in case of carriers were observed, but these results were not confirmed by power analysis.

IV.2.2. rs4844880 polymorphism

The allele frequency of the rs4844880 polymorphism did not differ between patients and controls. The SNP exerted a significant impact on BMI and weight gain during hormone replacement therapy. The BMI was significantly higher in rs4844880 carriers than in non-carriers (29.9 ± 3.7 and 25.1 ± 5.1 kg/m2, respectively; p=0.002, power: 95.5%). Carriers had significantly larger weight gain during hormone replacement therapy as compared to non-carriers (17.5 ± 9.87 and 4.05 ± 9.95 kg, respectively; p=0.02, power: 84%). In addition, the body weight adjusted substitution dose was lower in rs4844880 carriers as compared to non-carriers (0.278 and 0.371 mg/kg/day, respectively; p=0.018, power: 94.9%).

In addition, a positive effect of the polymorphism was observed in the annual density, T- and Z-score changes in the lumbar spine region. In the case of the density, this increase was significantly higher in carriers, while the T- and Z-score values showed an increasing tendency in case of carriers, and a decreasing tendency in case of non-carriers (0.024±0.03 vs. 0.001±0.03, p=0.017; 0.22±0.23 vs. -0.03±0.13, p=0.005; 0.26±0.22 vs -0.003±0.1 p=0.003; power: 92.1%, 90.7%, 96.2%).

Since 11βHSD2 does not metabolize dexamethasone, patients were divided into two groups based on the substitution therapy: patients with and without dexamethasone. No significant differences between age, total hydrocortisone equivalent or body weight adjusted substitution dose, fasting glucose concentration, serum cholesterol and triglyceride levels were observed between patients receiving HC alone and HC plus dexamethasone. However, higher BMI and higher weight gain during substitution therapy was found in carriers than in non-carriers of the rs4844880 polymorphisms who were treated with glucocorticoids other than dexamethasone (BMI, 31.2 ± 5 carrier and 23.8 ± 4.9 kg/m^2 non-carrier, respectively; weight gain during substitution therapy, 16.2 ± 12.5 carrier and 3 ± 8.9 kg non-carrier, respectively) but not in those receiving dexamethasone substitution therapy. It was also noted, that the polymorphic BclI allele was significantly overrepresented in patients treated with glucocorticoids other than dexamethasone as compared to those receiving dexamethasone substitution therapy (34.2 % and 18.3 %, respectively; p=0.03), which may modify the effect of the rs4844880 polymorphism.

In women patients, patients were divided into two subgroups depending on fertility, into pre- and post-menopausal groups based on age. Patients older than 50 years were considered as post-menopausal group. In the postmenopausal group, the rs4844880 polymorphism mainly associated with advantageous effect on the bone mineral density, while in the premenopausal

group, the body weight and BMI-influencing effects of the SNP became dominant, and there were no significant differences between the bone mineral density parameters.

IV.3. Case presentation - R714Q mutation of the *GR*

A 31-year-old woman presented at the 2nd Department of Medicine, Semmelweis University, Budapest because of infertility. Her history was unremarkable except unsuccessful attempts for pregnancy for the past 2.5 years. She had regular menstrual cycles since the age of 13 years. On clinical examination, she was normotensive and normokalemic without clinical signs of Cushing's syndrome or hyperandrogenism. Her height, BMI and glucose homeostasis and bone mineral density proved to be normal (height_170 cm, BMI 19.8 kg/m^2, fasting se. glucose: 5.0 mmol/l and HbA1c: 5.2%,), and galactorrhoea was absent. Family history was also unremarkable. Initial laboratory findings indicated an increased serum prolactin level (93 ng/ml; normal, 1.4-24 ng/ml), but this was due to macroprolactinemia (prolactin recovery after polyethylene glycol: PEG precipitation was 76%). Magnetic resonance imaging did not reveal any pituitary abnormality. Detailed hormone laboratory investigations of the patient suggested a partial resistance against glucocorticoids. During repeated measurements, serum cortisol levels in the morning were always elevated (between 35.4 and 26 µg/dl; normal: 8-25) while plasma ACTH concentration were slightly above the upper limit or within the normal range (between 65 and 28.5 pg/ml; normal: 7.2-63.3). Morning salivary cortisol levels (determined two times) were also elevated (1.36 and 1,13 µg/dl; normal <0.690) but salivary cortisol collected at midnight was within the reference range (0.21 and 0.23 µg/dl; reference range <0.430 µg/dl), which refers to maintained cortisol rhythm. A low dose (1 mg) overnight dexamethasone suppression test was performed twice, and showed an inadequate suppression of morning serum cortisol (10 and 15 µg/dl; reference range < 5 µg/dl). Repeated 24h urinary free cortisol (UFC) concentrations were between 280 and 513 nmol/day (reference range: 100-379). Serum DHEAS was slightly elevated or normal (342 and 163 µg/dl, reference range: 130-330), and serum androstendione was increased (344 ng/dl; reference range 80-280 ng/dl). GH, SHBG, TSH, fT4, LH, FSH, testosterone, progesterone and oestradiol levels were all normal.

After genetic counseling and written informed consent, Sanger sequencing of the coding region of the GR gene (NR3C1, NM_000176) was performed. A heterozygous missense mutation (c.2141G→A) resulting in a Arg714Gln change was identified in exon 8 of the GR gene, which was published first by Nader et al., and identified as pathogenic. After

identification of a pathogenic GR mutation, a family screening was indicated, and her 35-year-old, clinically healthy sister was also genetically tested, who has no fertility problems. The same mutation was found in the clinically healthy 35-year-old sister of the patients, who had normal steroid hormone levels. Other family members denied the clinical, genetic or hormonal screening.

The three-dimensional protein modeling showed that arginine at the position 714 is the member of helix 10 of the ligand binding domain (LBD) of the GR, which locates opposite side of the ligand binding pocket and relatively far from any known functional region. However, arginine has a large, positively charged side chain, which protrudes into a space created by helices 7–10, but glutamine has a smaller, uncharged side chain, which may release helix 10 from its original position, which may lead to further conformational changes in the ligand-binding pocket. Nader et al performed a complex functional testing of this mutation, which showed that the mutant LBD had an increased distance in root mean square deviation over the duration of the simulation compared to the wild type receptor, suggesting that the mutant structure binds the peptide with less affinity.

In addition, this mutation was not detected in more than 60 patients and controls tested either for glucocorticoid resistance or Cushing’s syndrome in our Laboratory. Moreover it was not present in commonly used genetic databases.

V. Conclusions

1. Homozygous carriers of the BclI. polymorphism of the GR gene have significantly higher BMI, than heterozygous carriers. In addition, the total hydrocortisone equivalent substitution dose was significantly lower in homozygous carriers, than in heterozygous or wild-type carriers.
2. The allele frequency of the N363S polymorphism of the GR gene was significantly higher in patients with Addison' disease compared to the healthy control group.
3. In the group of patients with Addison' disease, the disease appeared significantly earlier in carriers of the GR A3669G polymorphism compared to the non-carriers, irrespective of the carrier state of other SNPs.
4. In the group of patients with Addison' disease, carriers of the A3669G polymorphism had significantly higher serum ACTH levels at the diagnosis of the disease compared to the non-carriers.
5. The rs12086634 polymorphism showed a significant association with the annual change of the Z-score at the lumbal spine region, the annual decrease in the Z-score levels was significantly higher in carriers compared to the non-carriers.
6. The rs4844880 polymorphism exerted a significant impact on BMI and weight gain during hormone replacement therapy in Addison's disease. The BMI was significantly higher in carriers than in non-carriers. Carriers had significantly larger weight gain during hormone replacement therapy as compared to non-carriers. In addition, the body weight adjusted substitution dose was lower in carriers as compared to non-carriers.
7. Positive effect of the rs4844880 polymorphism was observed in the annual density, T- and Z-score changes measured at the lumbal spine region. In the case of the density, this increase was significantly higher in carriers, while the T- and Z-score values showed an increasing tendency in case of carriers, and a decreasing tendency in non-carriers.
8. Higher BMI and higher weight gain during substitution therapy was found in carriers of the rs4844880 than in non-carriers, who were treated with glucocorticoids other than dexamethasone, but not in those receiving dexamethasone substitution therapy.
9. In the postmenopausal group, the rs4844880 polymorphism mainly has advantageous effect on the bone mineral density, while in the premenopausal group,

the body weight and BMI-influencing effects of the SNP became dominant, and there were no significant differences between the bone mineral density parameters.

10. Using a direct DNA sequencing the R714Q mutation in the 8. exonic region of the GR gene, which was previously published by Nader et al was identified in a patient evaluated because of infertility. This mutation may be the etiologic factor of a 31-year-old female patient with clinically mild glucocorticoid resistance.

VI. Bibliography

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VII. List of publications not related to the Ph.D. theses

There is no such publication.