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The 83,557insA variant of the gene coding 11β-hydroxysteroid dehydrogenase type 1 enzyme associates with serum osteocalcin in patients with endogenous Cushing's syndrome

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ABSTRACT

Objective: The type 1 and type 2 isoenzymes of the 11β-hydroxysteroid dehydrogenase (HSD11B) play an important role in the prereceptor regulation of glucocorticoid bioavailability and action. The potential importance of gene variants coding HSD11B has not been previously evaluated in patients with endogenous hypercortisolism. The aim of the present study was to explore presumed associations between the 83,557insA variant of the *HSD11B1* gene and circulating hormone concentrations, bone turnover and bone mineral density (BMD) in patients with endogenous Cushing's syndrome (CS).
Patients and methods: Forty one patients with ACTH-producing pituitary adenomas (Cushing's disease—CD), 32 patients with cortisol-producing adrenal tumors (ACS) and 129 healthy control subjects were genotyped for the 83,557insA variant of the *HSD11B1* gene using restriction fragment length analysis. BMD was measured by dual-energy X-ray absorptiometry. Serum cortisol, ACTH, osteocalcin (OC) and C-terminal crosslinks (CTX) of human collagen type I (C-telopeptide) were measured by electrochemiluminescence immunoassay.
Results: No statistically significant differences were found in the allelic frequencies of the 83,557insA polymorphism among patients with CD, ACS and healthy controls. Among all patients with CS, heterozygous carriers of the 83,557insA had significantly higher serum OC as compared to non-carriers. Patients with ACS carrying the 83,557insA variant had higher plasma ACTH concentrations compared to non-carriers. The 83,557insA variant failed to associate with BMD in patients and controls.
Conclusions: Our present findings indicate that the 83,557insA variant of the *HSD11B1* gene may influence serum markers of bone turnover, but not BMD in patients with endogenous Cushing's syndrome.

1. Introduction

Type 1 and type 2 isoenzymes of the 11β-hydroxysteroid dehydrogenase play an important role in the prereceptor regulation of glucocorticoid bioavailability and action [1]. The type 1 enzyme is expressed in almost all tissues and controls the glucocorticoid action on a tissue specific manner, independent of circulating cortisol concentration [2]. The type 1 isoform (*HSD11B1*) is a NADP(H)-dependent bi-directional enzyme which primarily interconverts inactive cortisone to hormonally active cortisol by an oxoreductase activity [1,2]. It is highly expressed in glucocorticoid target tissues, facilitating glucocorticoid exposure to glucocorticoid receptor (GR) [3]. Contrary to type 1 isoenzyme, the expression of type 2 isoenzyme seems to be limited to cells and tissues dependent on mineralocorticoid action (renal tubules, colonic epithelia, etc.).

The importance of HSD11B1 enzyme in metabolic syndrome has been reviewed recently [4]. The well-known common features of metabolic syndrome and endogenous hypercortisolism underline its potential significance in the pathogenesis of Cushing's syndrome. On the other hand, several lines of evidence suggest that the sensitivity and cellular responses of various tissues to glucocorticoids, and even the setpoint of the hypothalamic–pituitary–adrenal axis are at least partially genetically determined [5–7]. Genetic variants of the HSD11B1 enzyme may have an important role among the various genetic factors contributing to the interindividual variability of the glucocorticoid response.

The human *HSD11B1* gene is localised to chromosome 1 (1q32-41) and consists of 6 exons [8]. To date, numerous associations have been reported between *HSD11B1* gene polymorphisms and clinical parameters or diseases, such as body mass, insulin resistance, hypertension, obesity, diabetes mellitus type 2, Alzheimer's

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disease and polycystic ovary syndrome [9–12]. In addition, functional analyses suggested that certain genetic variants reduce the transcriptional activity of the gene [13,14]. The 83,557insA genetic variant of the *HSD11B1* gene, located to the third intron, has been extensively studied and several associations have been described; it was associated with greater body mass, altered body composition and insulin resistance in overweight children [9]. Draper et al. constructed a triallelic digenic model suggesting that this variant might be involved in the inheritance of cortisone reductase deficiency [14]. In contrast with these findings, San Millán et al. did not find any association between the 83,557insA variant and polycystic ovary syndrome and there was no association with metabolic parameters in a Caucasian elderly population [15,16].

The functional importance of this variant of the *HSD11B1* gene has not been previously evaluated in patients with endogenous hypercortisolism. We hypothesized that the clinical variability and individual differences in the severity of Cushing's syndrome might be at least partially influenced by this genetic variant. Therefore, the aim of the present study was to explore presumed associations between the 83,557insA variant of the *HSD11B1* gene and circulating hormone concentrations, bone turnover and bone mineral density in patients with endogenous Cushing's syndrome.

2. Patients and methods

2.1. Patients and healthy controls

The allele frequency of the *HSD11B1* gene polymorphism was investigated in 73 patients with endogenous hypercortisolism, diagnosed at the 2nd Department of Medicine, Semmelweis University, Budapest. There were 41 patients with ACTH-producing pituitary adenomas (Cushing's disease—CD) and 32 patients with cortisol-producing adrenal tumors (ACS). At the time of diagnosis, all of the patients with CD and ACS had typical complaints and physical signs of endogenous hypercortisolism.

Genotype distributions of patients were compared to those of 129 healthy control subjects with no personal history of hypercortisolism or low bone mass. Known metabolic bone diseases, medical conditions and concurrent medications affecting bone mineral content represented exclusion criteria from the healthy control group.

The main demographic findings of patients and healthy control subjects are presented in Table 1. All patients and healthy controls were of Caucasian origin. The study was approved by the local Ethical Committee of Semmelweis University. Informed consent was obtained from all individuals participating in the study.

2.2. Endocrine investigations

All patients underwent a detailed clinical and hormonal evaluation. Serum cortisol concentrations at 0800 and 2400 h as well as after a low-dose dexamethasone suppression test (LDDST) (1 mg dexamethasone was given orally at 2400 h, and blood was drawn for serum cortisol measurement the next morning between 0800 and 0900 h) were measured. Blood samples for basal plasma ACTH concentration were taken between 0800 and 0900 h and the measurements were performed using immunochemiluminometric assay (Elecsys, F. Hoffmann-La Roche Ltd., Basel, Switzerland).

The diagnosis of CD was based on hormonal findings and pituitary imaging studies performed with magnetic resonance imaging (MRI) and/or computed tomography (CT). Bilateral inferior petrosal sinus catheterization was also performed in some patients. The diagnosis of ACS was based on hormonal findings and adrenal CT or MRI.

2.3. Bone mineral density measurement

Bone mineral density (BMD) of the lumbar spine (L1-4), proximal total femur and femoral neck, intertrochanteric and trochanteric subregions were measured by dual-energy X-ray absorptiometry (DEXA) using Hologic 4500C densitometer (Hologic, Waltham, MA, USA). Software version 9.03 was used. BMD was expressed as g/cm^2. BMD *Z*-scores were calculated according to the manufacturer's reference curves. NHANES III normative data were used as a reference database for femoral bone density measurements [17]. Quality control was maintained by daily scanning of Hologic anthropometric spine phantom. The coefficient of variation of BMD measurements with spine phantom over a period of 4 years was 0.0035 in our laboratory. To assess in vivo short-term precision of DEXA measurements, 30 patients were scanned twice with repositioning for duplicate postero-anterior lumbar spine and femur scans. Coefficients of variation of BMD measurements were 0.015 for lumbar spine, 0.018 for femoral neck, 0.017 for trochanteric region, 0.017 for intertrochanteric region and 0.012 for total femur.

2.4. Laboratory assessment of serum bone markers

Blood samples for measurement of biochemical markers of bone turnover were collected at 0800 h after an overnight fast. Patients were not instructed to keep any special diet. Blood samplings for measurements of bone formation and bone resorption markers were avoided after dexamethasone suppression tests and in patients receiving antiresorptive or other medications affecting bone turnover. Serum osteocalcin (OC) and serum C-terminal crosslinks of human collagen type I (C-telopeptide) (CTX) were measured with kits from Roche Laboratory according to the manufacturer's instructions. The Elecsys N-MID OC test uses two monoclonal antibodies, which recognize epitopes of the MID- and N-terminal fragment of OC. The Elecsys β-CrossLaps assay is specific for β-isomerised type I collagen fragments. According to the manufacturer, the lower and upper normal threshold values (5–95th percentile) of OC were 11–43 ng/mL and 15–46 ng/mL in healthy premenopausal and postmenopausal females and 14–42 ng/mL in healthy males. CTX concentrations were 0.299 ± 0.137 ng/mL and 0.556 ± 0.226 ng/mL for healthy premenopausal and postmenopausal women, respectively, and 0.304 ± 0.200 ng/mL for men. The analytical performances of serum CTX and OC measurements with the Roche Elecsys assay were as follows: detection range, 0.01–6.00 ng/ml and 0.5–300 ng/ml; within-run precision, 1.0–4.6 CV% and 0.5–1.1 CV%; total precision, 2.7–7.6 CV% and 1.1–1.6 CV%, respectively.

2.5. Analysis of the 83,557insA variant of the HSD11B1 gene

Total genomic DNA was isolated from peripheral blood leukocytes using a QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) and DNA Isolation Kit for Mammalian Blood (Mannheim, Germany; Indianapolis, IN, USA). The 83,557insA polymorphism was identified using restriction fragment length analysis (RFLP) [9]. DNA samples were amplified using forward and reverse oligonucleotide primers (F: 5′-AGACTACCCCCCAAAAAT-3′; R: 5′-TGTCCCTGTCCCACTTAC-3′). The generated 461/462 bp polymerase chain reaction (PCR) fragments were digested with the XcmlI restriction endonuclease (New England Biolabs GmbH, Frankfurt am Main, Germany). If the generated PCR product contained a cleavage site for the XcmlI enzyme, the reaction yielded fragments of 210 and 252 bp (Fig. 1.). The results of RFLP were validated with

Table 1
Demographic and hormonal findings in patients with endogenous Cushing's syndrome and healthy controls.

	Patients with CD	Patients with ACS	Healthy controls
Number of subjects	41	32	129
Female/male	35/6	27/5	94/35
Mean age, years	34.46 ± 12.30**,a	48.66 ± 13.60	49.38 ± 14.95
BMI, kg/m^2	30.53 ± 6.73[a]	29.74 ± 5.84[a]	26.47 ± 4.7
Plasma ACTH at 0800 h, pg/ml	142.04 ± 133.28**	5.34 ± 7.58	–
Serum cortisol at 0800 h, μg/dl	22.86 ± 11.32	19.48 ± 10.48	–
Serum cortisol at 2400 h, μg/dl	19.82 ± 13.27*	14.31 ± 11.47	–
Serum cortisol after low dose dxm, μg/dl	17.82 ± 9.62	14.89 ± 11.67	–

Reference ranges: plasma ACTH: 20–70 pg/ml; serum cortisol at 0800: 8–25 μg/dl; serum cortisol at 2400: <5 μg/dl; serum cortisol after low dose dxm <2.0 μg/dl. Results are presented as means ± SD. CD: Cushing's disease, ACS: adrenal Cushing's syndrome, BMI: body mass index, dxm: dexamethasone.
[a] $p < 0.01$ vs. healthy subjects.
* $p < 0.05$ vs. patients with adrenal Cushing's syndrome.
** $p < 0.01$ vs. patients with adrenal Cushing's syndrome.

direct sequencing (ABI PRISM® 3100 Genetic Analyzer) in all of the 83,557insA carriers, and the findings showed 100% coincidence with those obtained by the RFLP technique.

2.6. Statistical analysis

All statistical analyses were performed using Statistica package (version 7.0, Statsoft Inc., Tulsa, OK, USA). A value of $p < 0.05$ was considered to be significant. Normality of data distribution was analyzed by the Shapiro–Wilk's test. The differences in anthropometrical measurements, hormonal and osteodensitometric parameters and bone turnover markers between patient groups and healthy controls were evaluated with Student's *T*-test or Mann–Whitney rank sum test, based on data distribution. Genotype frequencies of the 83,557insA polymorphism of the HSD11B1 gene were compared between all patients with endogenous Cushing's syndrome and healthy controls with chi-square analysis. The association analyses between genotypes, clinical, hormonal and osteodensitometric parameters were evaluated using Student's *T*-test or Mann–Whitney rank sum test depending on normality. Analysis of covariance (ANCOVA) was applied to assess the association between bone markers and genotypes, using 83,577insA genotypes as a factor while age, gender and BMI as covariates. Gender was categorized as male, premenopausal female and postmenopausal female. Multiple testing corrections for pairwise comparison between 83,557insA genotypes and OC levels were performed by Bonferroni test. All groups were evaluated in connection with the Hardy–Weinberg equilibrium for the 83,557insA variant.

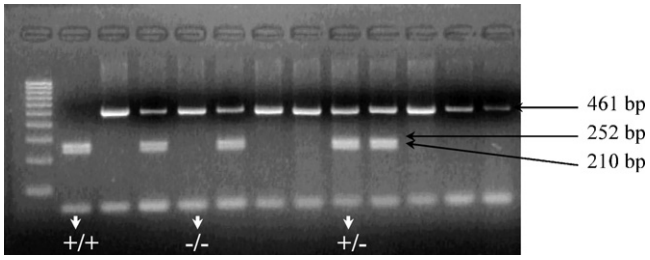

Fig. 1. Restriction fragment length analysis for the detection of the 83,557insA polymorphism. DNA samples were amplified with forward and reverse oligonucleotide primers. The generated 461/462 bp fragments were digested with the XcmII restriction endonuclease. If the generated PCR product contained a cleavage site for the XcmII enzyme, the reaction yielded fragments of 210 and 252 bp.

3. Results

3.1. Demographic findings, hormonal, clinical and osteodensitometric parameters

Age was similar in patients with ACS and healthy controls whereas patients with CD were younger than those with ACS or healthy controls ($p < 0.01$). Body mass index (BMI) of patients with endogenous Cushing's syndrome was higher than that of healthy subjects ($p < 0.01$). Serum cortisol concentrations at 2400 h ($p < 0.05$) were higher in patients with CD than in patients with ACS. The main demographic and hormonal findings are detailed in Table 1.

As expected, BMD and BMD *Z*-scores of the lumbar spine and femoral regions were significantly lower in patients with both CD and ACS than in healthy subjects. There were no statistically significant differences in BMD and BMD *Z*-score values measured at any site between patients with CD and ACS. Serum OC was significantly lower in both patient groups with endogenous hypercortisolism compared to healthy subjects ($p < 0.01$), but serum OC levels did not differ between patients with CD and ACS. No significant difference was observed in serum CTX between patients with CD, ACS and healthy controls (Table 2).

3.2. Allelic frequency of the 83,557insA polymorphism of the HSD11B1 gene in patients with endogenous Cushing's syndrome and in healthy controls

No statistically significant differences were found in the allelic frequency of the 83,557insA polymorphism among patients with CD, ACS or the combined group of patients with CD + ACS and healthy controls (Table 3). The distribution of the 83,557insA polymorphism was in Hardy–Weinberg equilibrium in all three groups.

3.3. Lack of correlations between the 83,557insA variant of the HSD11B1 gene and clinical parameters in healthy controls

The 83,557insA polymorphism of the *HSD11B1* gene was not associated with BMI, BMD, BMD *Z*-scores of the lumbar spine and femoral sites, serum CTX and OC concentrations in the healthy control population (data not shown).

3.4. Associations between 83,557insA polymorphism and clinical findings in patients with ACS

In patients with ACS, there were no statistically significant differences in serum cortisol concentrations at 0800 and 2400 h, and after LDDST between carriers and non-carriers of the 83,557insA variant. However, the polymorphic 83,557insA genotype was

Table 2
Bone mineral density and serum bone markers of patients with endogenous Cushing's syndrome and healthy controls.

	Patients with CD	Patients with ACS	Healthy controls
Lumbar spine BMD, g/cm^2	0.878 ± 0.127**	0.863 ± 0.117**	1.051 ± 0.183
Lumbar spine *Z*-score	−1.21 ± 1.27**	−1.05 ± 1.12**	−0.12 ± 1.13
Total femur BMD, g/cm^2	0.843 ± 0.117**	0.833 ± 0.132**	0.997 ± 0.147
Total femur *Z*-score	−0,66 ± 1.01**	−0.48 ± 1.09	+0.11 ± 1.10
Femoral neck BMD, g/cm^2	0.744 ± 0.105**	0.722 ± 0.119**	0.872 ± 0.114
Femoral neck *Z*-score	−0.67 ± 0.97**	−0.36 ± 1.10*	+0.15 ± 0.97
Intertrochanteric BMD, g/cm^2	0.988 ± 0.138**	0.989 ± 0.164**	1.161 ± 0.167
Intertrochanteric *Z*-score	−0.63 ± 0.89**	−0.41 ± 1.10	+0.11 ± 1.02
Trochanteric BMD, g/cm^2	0.622 ± 0.104**	0.631 ± 0.108**	0.763 ± 0.123
Trochanteric *Z*-score	−0.71 ± 1.13**	−0.54 ± 1.07*	+0.27 ± 1.16
Serum OC, ng/ml	10.21 ± 5.39**	14.38 ± 9.95**	25.18 ± 9.86
Serum CTX, ng/ml	0.47 ± 0.28	0.39 ± 0.22	0.44 ± 0.20

Data are expressed as means ± SD. CD: Cushing's disease, ACS: adrenal Cushing's syndrome.
* $p < 0.05$ vs. healthy controls.
** $p < 0.01$ vs. healthy controls.

Table 3
Genotype distribution and allelic frequency of 83,557insA polymorphism of the *HSD11B1* gene in patients with endogenous Cushing's syndrome and healthy subjects. CD: Cushing's disease, ACS: adrenal Cushing's syndrome.

	Patients with CD ($n = 41$)	Patients with ACS ($n = 32$)	Patients with CD + ACS ($n = 73$)	Healthy controls ($n = 129$)
83,557insA				
−/−	29 (70.7%)	23 (71.9%)	52 (71.2%)	86 (66.7%)
−/A	11 (26.9%)	9 (28.1%)	20 (27.4%)	40 (31.0%)
A/A	1 (2.4%)	0 (0%)	1 (1.4%)	3 (2.3%)
Polymorphic allele frequency	**0.16**	**0.14**	**0.15**	**0.18**

found to be associated with ACTH levels in patients with ACS; the 83,557insA heterozygotes had significantly higher (i.e. less suppressed) plasma ACTH concentrations compared to patients with the wild-type variant (7.38 ± 4.05 pg/ml vs. 4.81 ± 8.25 pg/ml, $p = 0.025$; healthy reference range: 20–70 pg/ml). Additionally, the 83,557insA carriers had smaller tumor size compared to non-carriers (28.44 mm ± 9.91 vs. 51.05 ± 38.53 mm, $p = 0.03$) (Table 4). There was no correlation between plasma ACTH level and tumor size in patients with ACS ($p = 0.47$, $r = 0.14$). In patients with CD, no statistically significant differences were found between the presence of the 83,557insA genotype and hormone concentrations (plasma ACTH levels, serum cortisol concentrations collected at 0800, 2400 h and after LDDST) as well as BMI.

3.5. Association of the 83,557insA polymorphism with serum bone markers but not with bone mineral density in patients with Cushing's syndrome

Among all patients with endogenous hypercortisolism carriers of the 83,557insA variant had significantly higher serum OC as compared to non-carriers (15.88 ± 10.24 ng/ml vs. 10.24 ± 5.87 ng/ml). Considering several potential predictors of bone metabolism in a covariance analysis, the association between the 83,557insA polymorphism and serum OC remained statistically significant after adjustment for age, gender and BMI. In contrast to serum OC, there was no association between the presence of 83,557insA allele and serum CTX concentrations in a univariate and in a covariance analysis (Tables 5 and 6).

The 83,557insA polymorphism failed to show any correlation with BMD and BMD *Z*-scores at the lumbar spine, proximal total femur and femoral subregions in patients with endogenous Cushing's syndrome. Furthermore, serum cortisol collected at 0800, 2400 h, and after LDDST was not statistically different between carriers and non carriers (Table 5).

4. Discussion

Prolonged exposure to excessively produced glucocorticoids results in the development of Cushing's syndrome, not infrequently presenting as a state of severe metabolic syndrome [18]. Convincing evidence suggests that the mild hypercortisolism present in metabolic syndrome may be accompanied by an increased glucocorticoid effect in peripheral tissues (fat, liver, etc.) and dysregulation of the hypothalamic–pituitary–adrenal axis [4,19]. The common characteristics of these two syndromes suggest that beyond the regulation of glucocorticoid secretion, the cellular effects of glucocorticoids should also be regulated, probably on an intracellular level [4]. The activity of the HSD11B1 enzyme and the

Table 4
Hormonal and clinical parameters according to 83,557insA genotypes in patients with adrenal Cushing's syndrome and Cushing's disease.

	Adrenal Cushing's syndrome			Cushing's disease		
	83,557insA non carriers	83,557insA carriers	*p*	83,557insA non carriers	83,557insA carriers	*p*
Plasma ACTH at 0800 h, pg/ml	4.81 ± 8.25	7.38 ± 4.05	0.025	143.66 ± 91.22	138.41 ± 204.15	0.11
Serum cortisol at 0800 h, μg/dl	19.62 ± 11.53	19.00 ± 6.58	0.73	24.55 ± 11.97	19.07 ± 9.00	0.20
Serum cortisol at 2400 h, μg/dl	15.63 ± 12.40	9.50 ± 5.39	0.17	19.36 ± 12.15	20.93 ± 16.20	0.80
Serum cortisol after low dose dxm, μg/dl	16.63 ± 12.51	9.43 ± 6.45	0.09	18.18 ± 9.22	16.94 ± 10.94	0.69
Δ serum cortisol, μg/dl	2.70 ± 8.65	9.57 ± 10.55	0.09	6.37 ± 7.98	2.77 ± 4.94	0.17
Mean age, years	45.78 ± 11.77	56.00 ± 15.87	0.05	36.59 ± 12.61	29.33 ± 10.24	0.09
Mean BMI, kg/m^2	29.92 ± 6.69	29.21 ± 1.95	0.79	31.14 ± 7.02	29.08 ± 6.03	0.22
Adrenal tumor size, mm	51.05 ± 38.53	28.44 ± 9.91	0.03			

Δ serum cortisol: decrease of serum cortisol after LDDST as compared to serum cortisol at 0800 h.

Table 5
Osteodensitometric parameters and serum bone markers according to 83,557insA genotypes in patients with endogenous Cushing's syndrome.

	83,557insA		
	Non carriers	Carriers	*p*
Lumbar spine BMD, g/cm^2	0.876 ± 0.129	0.860 ± 0.106	0.64
Lumbar spine *Z*-score	−1.13 ± 1.20	−1.16 ± 1.24	0.93
Total femur BMD, g/cm^2	0.841 ± 0.134	0.835 ± 0.097	0.873
Total femur *Z*-score	−0.60 ± 1.14	−0.52 ± 0.77	0.80
Femoral neck BMD, g/cm^2	0.743 ± 0.118	0.712 ± 0.091	0.33
Femoral neck *Z*-score	−0.47 ± 1.11	−0.66 ± 0.82	0.52
Intertrochanteric BMD, g/cm^2	0.981 ± 0.727	1.005 ± 0.126	0.42
Intertrochanteric *Z*-score	−0.62 ± 1.05	−0.30 ± 0.75	0.12
Trochanteric BMD, g/cm^2	0.627 ± 0.116	0.598 ± 0.071	0.35
Trochanteric *Z*-score	−0.59 ± 1.22	−0.74 ± 0.74	0.63
Serum CTX, ng/ml	0.43 ± 0.28	0.46 ± 0.20	0.44
Serum osteocalcin, ng/ml	10.24 ± 5.87	15.88 ± 10.24	0.027
Serum cortisol at 0800 h, μg/dl	22.28 ± 11.91	19.05 ± 8.00	0.46
Serum cortisol at 2400 h, μg/dl	17.65 ± 12.28	16.89 ± 14.31	0.63
Serum cortisol after low dose dxm, μg/dl	17.48 ± 10.73	14.02 ± 9.97	0.19

sensitivity of the glucocorticoid receptor may have an important role in the determination of the variability of tissue specific glucocorticoid response as well as the severity and clinical variability of these syndromes [3]. The associations between genetic variants of the glucocorticoid receptor gene and some of the metabolic parameters have been previously described [6,20,21], while the impact of glucocorticoid receptor gene variants on bone turnover and bone mineral density in patients with endogenous glucocorticoid excess syndromes was recently published by our group [22]. In addition, a recent study showed significant associations between some of the genetic variants of the *HSD11B1* gene and reduced vertebral fracture risk in postmenopausal osteoporosis, but the 83,557insA variant was not investigated and bone markers were not determined [23].

The main new findings of our study are that among patients with endogenous Cushing's syndrome, carriers of the 83,557insA variant of the *HSD11B1* gene had significantly higher serum OC level compared to non-carriers, while the BMD failed to show significant differences between carriers and non-carriers. This discrepancy between serum OC and BMD is not entirely unexpected, because serum OC is known to be a more sensitive marker of glucocorticoid-induced changes of bone metabolism than BMD assessed by DEXA measurements [24,25]. Our findings regarding the association of 83,557insA polymorphism with serum OC levels in patients with endogenous Cushing's syndrome but not in healthy subjects may represent a further example demonstrating that the functional impact of a given genetic polymorphism may vary depending on hormonal milieu. In addition, our present study showed that patients with ACS carrying the 83,557insA polymorphic allele had significantly less suppressed (i.e. higher) ACTH levels and a significantly smaller adrenal tumor size compared to patients not carrying the 83,557insA polymorphism. Because the intronic region containing the site of the 83,557 polymorphism may act as an enhancer of the *HSD11B1* expression and the presence of the 83,557insA variant results in a reduced transcriptional activity of the *HSD11B1* gene [14], it is possible that the higher OC and ACTH concentrations as well as the smaller adrenal tumor size found in our 83,557insA carriers could be attributed to a reduced HSD11B1 enzyme activity, although a cause and effect relationship between these parameters is difficult to explain. The possibility of a decreased HSD11B1 activity in osteoblasts resulting in a less suppressed serum OC in 83,557insA carriers is supported by the presence of HSD11B1 in human osteoblasts [26,27]. The *HSD11B1* gene is also expressed in pituitary tumors, especially in corticotroph adenomas [28,29] and in the normal human pituitary gland [30] and it may, therefore, modulate the inhibitory effect of cortisol on ACTH secretion. Similarly, *HSD11B1* expression has been detected both in normal human adrenal cortex and in adrenal adenomas as well [31,32], although its potential role in adrenocortical cell proliferation and/or secretion has not been investigated. Clearly, further investigations are needed to explore the exact role of the HSD11B1 in osteoblasts, corticotrophs and adrenocortical cells as well as to elucidate the mechanism(s) leading to the observed differences between the 83,557insA carriers and non-carriers.

Table 6
Serum bone markers according to 83,557insA genotypes adjusted for age, gender and BMI by covariance analysis in patients with endogenous Cushing's syndrome and healthy subjects. (Gender was categorized as male, premenopausal female and postmenopausal female.) *p*-Values of pairwise comparison between 83,557insA genotypes after multiple testing correction using Bonferroni post hoc test: 83,557insA non carriers vs. 83,557insA carriers: $p = 0.015$.

	Healthy controls		Endogenous Cushing's syndrome	
	Model included	*p*	Model included	*p*
Serum CTX	83,557insA	0.727	83,557insA	0.953
	Age	0.237	Age	0.189
	Gender	0.828	Gender	0.423
	BMI	0.097	BMI	0.482
Serum OC	83,557insA	0.839	83,557insA	*0.004*
	Age	0.206	Age	0.015
	Gender	0.114	Gender	0.024
	BMI	0.059	BMI	0.070

The low number of patients with endogenous Cushing's syndrome represents the most important limitation of the present study. Another, probably less important limitation is that we used only one bone formation and one bone resorption marker.

In conclusion, our present findings indicate that the 83,557insA variant of the *HSD11B1* gene may influence serum markers of bone turnover, but not BMD in patients with endogenous Cushing's syndrome. In addition, our results show that this variant is associated with a less suppressed plasma ACTH and a smaller adrenal tumor size in patients with ACS. The precise explanation for the latter finding remains to be investigated.

Conflict of interest

All authors have nothing to declare.

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