Modulatory effect of *Bcl*I GR gene polymorphisms on the obesity phenotype in Brazilian patients with Cushing's disease

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OBJECTIVES: Patients with Cushing's disease exhibit wide phenotypic variability in the severity of obesity, diabetes and hypertension. In the general population, several glucocorticoid receptor genes (*NR3C1*) and *HSD11B1* polymorphisms are associated with altered glucocorticoid sensitivity and/or metabolism, resulting in an increased or reduced risk of an adverse metabolic profile. Our aim was to analyze the association of *NR3C1* and *HSD11B1* gene variants with the severity of some clinical and hormonal features of Cushing's disease.

METHODS: Sixty-four patients presenting with Cushing's disease were diagnosed based on adrenocorticotrophic hormone levels, high-dose dexamethasone suppression tests and/or inferior petrosal sinus sampling and magnetic resonance imaging. The A3669G, ER22/23EK, N363S *Bcl*I-*NR3C1* and *HSD11B1*-rs12086634 variants were screened.

RESULTS: The *Bcl*I, *HSD11B1*-rs12086634 and A3669G variants were found in 36%, 19.5% and 14% of alleles, respectively. The N363S and ER22/23EK polymorphisms were identified in heterozygosis once in only two patients (1.5% of alleles). There were no differences in the weight gain or prevalence of diabetes and hypertension in the patients carrying the abovementioned alleles compared to the wild-type carriers. Interestingly, the mean body mass index (BMI) of the *Bcl*I carriers was significantly higher than the non-carriers (34.4 ± 7 kg/m^2 *vs.* 29.6 ± 4.7 kg/m^2, respectively). None of the polymorphisms were associated with the basal adrenocorticotrophic hormone, FU levels or F level after dexamethasone suppression testing.

CONCLUSION: Although Cushing's disease results from increased glucocorticoid secretion, we observed that interindividual variability in the peripheral glucocorticoid sensitivity, mediated by the glucocorticoid receptor, could modulate the obesity phenotype.

KEYWORDS: Glucocorticoid Receptor Polymorphisms; *HSD11B1* Polymorphism; Cushing's Disease; Obesity.

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INTRODUCTION

Cushing's disease (CD) comprises a wide range of clinical and biochemical features that result from prolonged and inappropriate glucocorticoid exposure. Elevated glucocorticoid levels can result from pituitary adrenocorticotrophic hormone (ACTH) excess, which is frequently associated with a pituitary adenoma (1).

The estimated incidence of CD ranges from 0.7 to 2.4 cases per million individuals per year and typically affects adults aged between 20 and 50 years of age (2). The clinical manifestations of CD include weight gain, hypertension, diabetes, cardiovascular disease and thromboembolic events, which significantly increase the mortality rate of CD patients compared to the normal population (3,4). The cure for CD or its remission frequently reduce mortality rates but do not completely eliminate cardiovascular risks, even after the normalization of cortisol (F) secretions (5). The severity of these comorbidities is at least partially determined by an interindividual variation in the peripheral glucocorticoid (GC) sensitivity, which is mediated by glucocorticoid receptor gene polymorphisms (6).

Several GR polymorphisms have been identified, but only a few are functionally relevant. The *Bcl*I, N363S, ER22/23EK

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and A3669G alleles have been associated with changes in GC sensitivity as well as with altered cortisol levels (6). In the general population, the N363S and *Bcl*I polymorphisms exhibit positive associations with the increased prevalence of abdominal obesity, hypertension and coronary artery disease (6–8). In addition, because of their increased GC sensitivity, healthy subjects carrying either the N363S or *Bcl*I polymorphisms exhibit lower serum basal cortisol levels as well as greater serum cortisol reduction after dexamethasone administration (6). In contrast, the ER22/23EK and A3669G polymorphisms are associated with decreased GC sensitivity and relative GC resistance. Recent studies have demonstrated that the presence of the ER22/23EK polymorphism is associated with a favorable metabolic profile and body composition and a smaller decrease in cortisol levels after a dexamethasone suppression test (DST) (6).

In the context of the associations between GR variants and an adverse metabolic profile in the general population, two recent studies have evaluated the effect of these polymorphisms on the clinical manifestations of CD (9). The first study of a cohort comprising 35 patients observed that the *Bcl*I carriers exhibited increased skeletal sensitivity to GC compared to wild-type carriers (10). The second study, of a similar sample size, observed that the A3669G carriers exhibited a lower prevalence of type 2 diabetes compared with wild-type carriers (9).

However, the interindividual variability in the peripheral sensitivity to glucocorticoids can be influenced by other factors in addition to GR activity, including cortisol regeneration by the 11β-hydroxysteroid dehydrogenases (11). A polymorphism located in the third intron of the *HSD11B1* gene causes lower transcriptional activity in in vitro studies (12) and has been associated with lower intracellular cortisol levels, particularly in adipose and liver tissues, which are protective features against developing metabolic syndrome. Consequently, we hypothesized that the severity of obesity in CD patients could be genetically influenced by the *HSD11B1* polymorphism.

The aim of the present study was to evaluate the association between the polymorphisms involved in GC action (GR *Bcl*I, N363S, ER22/23EK and A3669G) and peripheral metabolism (*HSD11B1* rs12086634) on the severity of some clinical and hormonal manifestations in a large cohort of CD patients who were followed at a single center. We observed that in addition to manifesting CD, the severity of obesity in CD patients could be modulated by genetic factors because the *Bcl*I carriers exhibited higher body mass indices (BMIs) than non-carriers.

METHODS

Subjects

The study was approved by the Ethical Committee of the Hospital das Clínicas da Faculdade de Medicina da Universidade de Sao Paulo, and informed consent was obtained from all the participants.

The study included 64 patients (51 females and 13 males) with a mean age of 25.2±5.2 years who underwent first-time transphenoidal surgery to treat CD between 1989 and 2011 at our institution (Hospital das Clínicas da Faculdade de Medicina da Universidade de Sao Paulo, Endocrinology Department, Sao Paulo, Brazil). Data regarding the preoperative diagnosis (clinical features, laboratory data and imaging studies) were retrospectively recorded. Genomic DNA samples were available from all 64 patients.

Diagnosis of CD

CD was diagnosed using the currently accepted standard criteria (2,13). At least two elevated 24-h urine F excretion levels and/or failing to suppress serum F during a low-dose overnight dexamethasone suppression test were used to establish endogenous hypercortisolemia in all patients. High-dose dexamethasone suppression and/or a desmopressin test were also performed to evaluate the ACTH and F and responses. To evaluate the presence of pituitary adenomas in 2005, computed tomography (CT) was used, and after that year, magnetic resonance imaging (MRI) was used. When necessary, inferior petrosal sinus sampling tests were used to establish the pituitary origin (14).

Tumor size

Tumor size was classified based on preoperative radiologic images (MRI/CT) and intraoperative findings: microadenoma (tumors ≤10 mm in the maximal diameter) or macroadenoma (tumors >10 mm).

Clinical, anthropometric and laboratory measurements

All of the patients underwent physical examinations to obtain clinical, hormonal and anthropometric measurements. Obesity was defined as having a BMI>30 kg/m^2, and overweight individuals were characterized by a BMI between 25 and 30 kg/m^2. Patients were defined as having elevated blood pressure if their blood pressure was >135/85 mmHg or if they were currently being treated for hypertension. Elevated glucose levels were defined as values >110 mg/dL (6.16 mmol/liter).

Plasma F concentrations were measured at 08:00 h and after a dexamethasone suppression test. One milligram or 8 mg of dexamethasone were given orally at midnight, and blood was drawn for plasma F measurements the next morning between 8:00 a.m. and 9:00 a.m. Histopathological analyses performed after the transphenoidal surgery confirmed the presence of an ACTH-producing pituitary adenoma.

ACTH was measured using RIA and IRMAs. Serum and urinary cortisol were measured using IRMAs.

GR genotyping

DNA samples from all patients were obtained from peripheral blood leukocytes using a salting out procedure.

PCR amplification of the glucocorticoid receptor gene regions was performed using primer sequences and amplification conditions, as previously described (15,16).

The ER22/23EK allele of GR comprises two linked single-nucleotide variations that are separated by one base pair in exon 2. The first substitution at nucleotide position 198 is silent, changing codon 22 from GAG to GAA, both coding for a glutamic acid (E). The second mutation changes codon 23 at nucleotide position 200 from AGG to AAG, causing a change from arginine (R) to lysine (K). The N363S polymorphism changes codon 363 of exon 2 at nucleotide position 1220 from AAT to AGT and results in an asparagine (N) to serine (S) amino acid change. The A3669G (also called 9β polymorphism) is located in the 3′ untranslated region of exon 9β at nucleotide position 3669

and results in an A to G substitution. These GR alleles were screened by sequencing.

The *HSD11B1* rs12086634 comprises an A insertion at the 83,557 position in intron 3 of the *HSD11B1* gene and was genotyped, as previously described (17). Amplified products were sequenced using the Big Dye Terminator Sequencing Kit™ (Applied Biosystems, Inc., Foster City, CA, USA) and submitted to capillary electrophoresis using an ABI PRISM 3100 sequencer (Applied Biosystems, Inc.). Sequence traces were analyzed using Sequencher (version 4.5, build 1416, Gene Codes Corp., Ann Arbor, Michigan).

The *Bcl*I polymorphism of GR results in an intronic C to G change, 646 nucleotides downstream of exon 2. This polymorphism was screened using allele-specific PCR, as previously described (18). The results of the allele-specific PCR were confirmed by direct sequencing in 20 patients.

Statistical analysis

The comparison of genotypic frequencies between the different groups of patients and between males and females was performed using the χ^2 test. The normal distribution of all continuous variables was tested, and some variables were logarithmically transformed. At baseline, independent t-tests for the independent groups were applied to compare the continuous variables. The results are reported as the means ± SD. These analyses were also performed with adjustments for age and sex by multivariable modeling. $p<0.05$ was considered significant.

The Hardy–Weinberg equilibrium for the *Bcl*I, A3669G and *HSD11B1* variants was calculated. Considering the small number of homozygous carriers of GR and *HSD11B1* polymorphisms, the homozygous and heterozygous subjects were analyzed as a single group, which was defined as 'carriers.'

Statistical analysis was performed using the software SigmaStat, version 3.5 for Windows (Systat Software, Point Richmond, CA).

RESULTS

Clinical and hormonal characteristics of CD patients

The clinical and hormonal baseline data of the 64 CD patients are described in Table 1. The patients exhibited detectable ACTH levels. Among the patients included in this study, 47 exhibited corticotropic microadenomas, and 11 exhibited macroadenomas, which were diagnosed with pituitary MRIs. For six patients, the pituitary lesion was unidentified, and catheterism of the inferior petrosal sinus confirmed the pituitary origin of the ACTH secretion.

Obesity was observed in 53% of patients (n = 34, 27 females, seven males), and an overweight phenotype was observed in 25% of the patients (n = 16, 12 females, four males). Hypertension was observed in 37.5% of the patients (n = 24, 18 females, six males), and the fasting plasma glucose level exceeded 110 mg/dL (6.16 mmol/L) in 28% of patients (n = 18, 15 females, three males).

The clinical and biochemical features of the patients exhibiting microadenomas were compared with those of the patients exhibiting macroadenomas. BMI, weight gain, blood pressure, fasting glucose levels, basal serum ACTH and cortisol levels, and serum ACTH and cortisol levels after DST tests did not differ significantly ($p>0.05$, data not shown). However, the patients with microadenomas exhibited higher total urine cortisol levels compared with patients with macroadenomas (1,203 ± 794.4 μg/24 h *vs.* 761.2 ± 650.7 μg/24 h, respectively. $p = 0.01$).

Table 1 - The clinical and hormonal characteristics of 64 CD patients.

Variables	Patients
Age at diagnosis, years	31.8 ± 12.2
BMI, kg/m^2	33.2 ± 6.9
Disease duration, years	4.2 ± 3.7
Weight gain, kg	24.8 ± 17.7
Systolic blood pressure, mmHg	140 ± 20.7
Diastolic blood pressure, mmHg	91.2 ± 13.9
Fasting glucose, mg/dL	106.9 ± 42.9
Morning serum cortisol, μg/dl	25.9 ± 16.5
Basal ACTH, μg/dl	79 ± 69.7
F after 1 mg DST, μg/dl	15.3 ± 11.9
F after 8 mg DST, μg/dl	9.7 ± 8.5
UFC, μg/24 h	1,081 ± 745
Image	
Microadenoma, n° (%)	47 (73.4)
Macroadenoma, n° (%)	11 (17.2)
Unidentified, n° (%)	6 (9.4)

Values are reported as the means ± SD. BMI, Body mass index; DST, Dexamethasone suppression test; UFC, Urine free cortisol; F, Cortisol.

Allelic frequencies of the GR and *HSD11B1* polymorphisms

The *Bcl*I, A3669G and *HSD11B1*-rs12086634 polymorphisms were in Hardy-Weinberg equilibrium, which was not calculated for the N363S and ER22/23EK polymorphisms because of the low frequency of the polymorphic genotypes.

Among the GR polymorphisms, the *Bcl*I polymorphism was observed in 36% of the alleles (38 heterozygote and four homozygote carriers), and the A3669G polymorphism was observed in 14% of alleles (16 heterozygote carriers and one homozygote carrier). The N363S and ER22/23EK polymorphisms were identified in heterozygosis once in only two patients (1.5% of alleles). The *HSD11B1* rs12086634 allele was observed in 19.5% of alleles (17 heterozygote and four homozygote carriers).

There was no significant difference in the *Bcl*I polymorphism frequencies between obese and non-obese CD patients [n = 26 (76.5%) *vs.* n = 15 (52.6%), respectively, $p>0.05$] nor was there a difference observed between the patients with and without diabetes [n = 10 (55.5%) *vs.* n = 30 (73.5%), respectively, $p>0.05$] and those with and without hypertension [n = 22 (75.9%) *vs.* n = 20 (59%), respectively, $p>0.05$].

With respect to the A3669G allelic frequencies, no significant differences were observed in the patients with and without obesity [n = 9 (26%) *vs.* n = 10 (33%), respectively, $p>0.05$], those with and without diabetes [n = 05 (28%) *vs.* n = 12 (26%), respectively, $p>0.05$] or those with and without hypertension [n = 6 (21%) *vs.* n = 11 (31%), respectively, $p>0.05$].

There were no differences in the rs12086634 frequencies between obese and non-obese patients [n = 12 (35%) *vs.* n = 9 (30%), respectively, $p>0.05$], patients with and without diabetes [n = 07 (39%) *vs.* n = 14 (30%), respectively, $p>0.05$] or with and without hypertension [n = 9 (32%) *vs.* n = 12 (33%), respectively, $p>0.05$]. However, a higher frequency of this polymorphism was observed in the patients with a macroadenoma compared with the patients with a microadenoma [n = 5 (83.3%) *vs.* n = 7 (26%), respectively, $p = 0.04$].

Table 2 - Comparison of the clinical and hormonal features of CD patients carrying the *Bcl*I and wild-type alleles.

Features	Wild Type (n = 22)	*Bcl*I carriers (n = 42)	*p*-value
Age at diagnosis, years	32.2 ± 10.8	31.5 ± 12.5	0.773
Disease duration, years	3.6 ± 3.7	4.5 ± 3.8	0.185
BMI, kg/m^2	29.6 ± 4.7	34.4 ± 7	*0.012*
Weight gain, kg	20.2 ± 10.1	25 ± 16.7	0.445
Systolic blood pressure, mmHg	135.3 ± 15.2	142.5 ± 22	0.256
Diastolic blood pressure, mmHg	90 ± 10.9	91.9 ± 15.4	0.815
Fasting glucose, mg/dL	119 ± 49.3	98.7 ± 35.2	0.086
Morning serum cortisol, μg/dl	26.3 ± 16.7	26.1 ± 16.7	0.845
Basal ACTH, μg/dl	93.7 ± 83.7	73.3 ± 64	0.315
F after 1 mg DST, μg/dL	15.2 ± 14.2	15.4 ± 11.2	0.963
F after 8 mg DST, μg/dL	9 ± 7.6	10.2 ± 9.3	0.915
UFC, μg/24 h	1,338 ± 1,143	1,011 ± 506	0.683
Image			
Microadenoma, n° (%)	17 (77.3)	30 (71.5)	0.837
Macroadenoma, n° (%)	3 (13.6)	8 (19)	0.844
Not identified, n° (%)	2 (9.1)	4 (9.5)	0.692

Values are reported as the means ± SD. BMI, Body mass index; DST, Dexamethasone suppression test, DST; Urine free cortisol, UFC; F, Cortisol.

The influence of polymorphisms on the clinical and hormonal characteristics of CD patients

GR polymorphisms

A comparison of the clinical and laboratory data between carriers and non-carriers of the *Bcl*I polymorphism is shown in Table 2.

*Bcl*I carriers exhibited higher BMIs compared to non-carriers (34.4 ± 7 kg/m^2 and 29.6 ± 4.7 kg/m^2, respectively, $p = 0.01$). Although *Bcl*I carriers exhibited higher blood pressure and greater weight gain, these differences did not reach the level of significance ($p > 0.05$, Table 2).

Significant differences between the *Bcl*I carriers and the non-carriers were not observed with respect to basal morning cortisol, ACTH, UFC levels or cortisol levels after 1-mg and 8-mg-DSTs (Table 2, $p > 0.05$).

Table 3 shows the clinical and laboratory data of the A3669G and wild-type carriers. There were no significant differences in the BMI, blood pressure, fasting glucose or weight gain between the patients carrying the A3669G and those carrying the wild-type allele ($p > 0.05$). We also compared the clinical and laboratory data of seven patients carrying both *Bcl*I and A3669G polymorphisms with those carrying the wild-type allele, and no significant differences were detected (data not shown).

There was no difference in the disease duration among the *Bcl*I, A3669G and wild-type carriers.

HSD11B1 gene polymorphism (rs12086634)

The clinical and laboratory data of the *HSD11B1* rs12086634 and wild-type carriers are shown in Table 4. There were no statistically significant differences in the clinical, laboratory or metabolic data or in the duration of the disease between patients carrying the *HSD11B1* rs12086634 allele and those with the wild-type allele ($p > 0.05$). However, when considering the co-expression of the *HSD11B1* rs12086634 and *Bcl*I polymorphisms (seven patients), the BMI was higher in the group carrying both polymorphisms compared with the wild type carriers (35.5 ± 6.7 kg/m^2 *vs.* 30.2 ± 5.1 kg/m^2, respectively, $p = 0.04$). No differences were observed in the other parameters analyzed (data not shown, $p > 0.05$). None of the patients exhibited co-expression of the *HSD11B1* rs12086634 and A3669G polymorphisms.

Table 3 - Comparison of the clinical and hormonal features of CD patients carrying the A3669G and wild-type alleles.

Features	Wild Type (n = 47)	A3669G carriers (n = 17)	*p*-value
Age at diagnosis, years	31.9 ± 11.1	31.3 ± 13.7	0.925
Disease duration, years	4.3 ± 3.8	4 ± 3.6	0.803
BMI, kg/m^2	32.7 ± 6.1	33.4 ± 8.2	0.720
Weight gain, kg	23 ± 15	24.2 ± 14.6	0.712
Systolic blood pressure, mmHg	142.2 ± 21.4	135 ± 19.5	0.209
Diastolic blood pressure, mmHg	91.5 ± 13.7	90.7 ± 15.4	0.816
Fasting glucose, mg/dL	104.1 ± 39.5	108.9 ± 46.2	0.847
Morning serum cortisol, μg/dl	23 ± 10.5	32.2 ± 25.4	0.327
Basal ACTH, μg/dl	81.3 ± 77.1	73.1 ± 44	0.708
F after 1 mg DST, μg/dL	15.4 ± 11.7	15.3 ± 12.8	0.965
F after 8 mg DST, μg/dL	10.8 ± 8	8.1 ± 9.9	0.303
UFC, μg/24 h	1,085 ± 679	1,169 ± 970	0.858
Image			
Microadenoma, n° (%)	33 (70.2)	14 (82.4)	0.515
Macroadenoma, n° (%)	10 (21.3)	1 (5.8)	0.286
Not identified, n° (%)	4 (8.5)	2 (11.8)	0.9

Values are reported as the means ± SD. BMI, Body mass index; DST, Dexamethasone suppression test; UFC, Urine free cortisol; F, Cortisol.

Table 4 - Comparison of the clinical and hormonal features of CD patients carrying the *HSD11B1* rs12086634 and wild-type alleles.

Features	Wild Type (n = 43)	rs12086634 carriers (n = 21)	*p*-value
Age at diagnosis, years	30.9 ± 12.8	33 ± 9.2	0.532
Disease duration, years	4.1 ± 3.7	4.5 ± 4	0.770
BMI, kg/m^2	32.3 ± 7	33 ± 6.7	0.715
Weight gain, kg	25.9 ± 16.1	18 ± 10.1	0.100
Systolic blood pressure, mmHg	139.4 ± 20.6	141.8 ± 22.1	0.679
Diastolic blood pressure, mmHg	90.3 ± 14	93.2 ± 14	0.496
Fasting glucose, mg/dL	103.5 ± 41.4	110.1 ± 41.4	0.410
Morning serum cortisol, μg/dl	26.8 ± 17.8	24.7 ± 13.4	0.663
Basal ACTH, μg/dl	72.7 ± 4.4	93.8 ± 107.7	0.723
1 mg DST, μg/dL	14.3 ± 11	17.6 ± 14	0.419
8 mg DST, μg/dL	8 ± 8.4	10.7 ± 8.4	0.546
UFC, μg/24 h	1,081 ± 684	1,167 ± 920	0.768
Image			
Microadenoma, n° (%)	34 (79.1)	13 (61.9)	0.246
Macroadenoma, n° (%)	4 (9.3)	7 (33.3)	0.041
Not identified, n° (%)	5 (11.6)	1 (4.7)	0.687

Values are reported as the means ± SD. BMI, Body mass index; DST, Dexamethasone suppression test; UFC, Urine free cortisol; F, Cortisol.

DISCUSSION

It is widely recognized that CD patients are predisposed to developing obesity and other metabolic abnormalities, including visceral obesity, hypertension and insulin resistance (19), which increases their cardiovascular risk and contributes to the high mortality rates associated with this disease (20). These metabolic abnormalities have been associated with the effects of long-term GC overexposure, which inhibits the immune and pro-inflammatory responses by suppressing the synthesis of cytokines and inflammatory mediators (21). In CD patients, the increased cardiovascular risk is not only observed during the active phase of the disease but may be observed for a long time after the disease remission.

Although chronic GC exposure is associated with a worse metabolic profile (13), the frequencies of these comorbidities differ among CD patients and are not related to the disease duration. This lack of correlation suggests the presence of other variables that may modulate the clinical manifestations.

Recently, in a cohort comprising 30 patients, tumor size was reported to affect the clinical manifestation of CD patients; the patients with microadenomas exhibited higher blood pressure compared to those with macroadenomas (22). In our series, we did not observe differences between the metabolic profiles of patients with micro- and macroadenomas nor did we observe differences in the basal serum ACTH and cortisol levels, even after DST. In contrast to the results previously described in two series comprising 18 and 20 macroadenomas (23,24), we observed higher total urine cortisol levels among the patients with microadenomas compared with those with macroadenomas. Most likely our discordant results could be related to the sample size effect.

In addition to the effect of tumor size, the peripheral GC sensitivity can play an important role in the modulatory effect of cortisol exposure (26). Several studies have demonstrated the influence of GR polymorphisms on glucocorticoid sensitivity and peripheral action (6,27). In the general population, the *Bcl*I polymorphism has been associated with enhanced GC sensitivity and consequently with a worse metabolic profile, including high blood pressure, waist to hip circumference, BMI, hyperinsulinemia, impaired glucose metabolism and dyslipidemia (9,28). In our study, we observed a positive association between the *Bcl*I carrier status and an elevated BMI, which is in line with previous studies that found a significant association between this polymorphism and obesity in the general population (29–32). A recent study in a series of 52 patients with Cushing's syndrome did not identify any influence of *Bcl*I polymorphisms on the patients' metabolic profiles (9). Note that this latter study enrolled patients with different Cushing's etiologies: 38 had CD, and 14 patients exhibited adrenal Cushing's syndrome (ACS).

The influence of the *Bcl*I polymorphism in the prevalence of metabolic complications has also been demonstrated in patients with adrenal incidentalomas without evidence of overt hypercortisolism. An increased frequency of arterial hypertension was observed in homozygous *Bcl*I patients (33). Although in our cohort the *Bcl*I carriers tended to develop high blood pressure, this tendency did not reach the level of significance. However, in our study, only four patients were *Bcl*I homozygous carriers.

The A3669G polymorphism of the GR gene has been related to the increased expression and stability of the GR-β isoform in vivo, which does not bind efficiently to GCs or activate GC-response genes. In addition, the GR-β isoform acts as a dominant negative inhibitor of the active GR-α isoform, leading to a relative GC resistance (6). This polymorphism has been associated with increased levels of inflammatory parameters and, consequently, with an increased risk of myocardial infarction and cardiovascular heart disease in homozygous carriers (34), but an association between the A3669G polymorphism and a favorable lipid profile in men and a decreased waist-hip ratio in women were also implicated (35). In a series of 52 patients with Cushing's syndrome, this variant was observed to act as a protective factor against the developing diabetes (9). In contrast to this study, we did not observe differences in the clinical, hormonal or anthropometric features between the A3669G carriers and non-carriers in our cohort.

In humans, 11β-HSD1 expression has been observed to increase in the adipose tissue of obese patients (36), and the role of *HSD11B1* gene polymorphisms in the prevalence of metabolic syndrome has been explored (17,37,38). A frequent polymorphism, rs12086634, accounts for decreased 11β-HSD1 transcriptional activity in vitro (12) and suppressed

intracellular cortisol levels; it acts as a protective factor against the features of the metabolic syndrome.

To the best of our knowledge, this study is the first to evaluate the role that the *HSD11B1* gene polymorphism (rs12086634) plays in the clinical, anthropometric and hormonal profiles of CD patients. Nevertheless, contrary to our expectations, we did not find any association among the *HSD11B1* genotype, clinical and hormonal profiles of CD patients (Table 4). However, only four patients were homozygous for this variant. Interestingly, the frequency of this polymorphism was significantly higher in the patients with macroadenomas compared with those with microadenomas (Table 4). Given that macroadenomas are often associated with higher serum ACTH levels compared with microadenomas (24), we speculated that this result could be explained by the fact that the *HSD11B1* rs12086634 reduces 11β-HSD1 transcriptional activity, consequently impairing the cortisol regeneration in peripheral tissues and resulting in the compensatory activation of ACTH secretion.

Our study has some limitations. First, our sample size was limited because we tried to select a homogenous cohort and only enrolled patients with pituitary CD origins. Second, this study was retrospective, and glucose tolerance tests were not available for several patients at that time. Therefore, the role of GR polymorphisms in diabetes frequency could not be evaluated precisely.

In conclusion, this study is the first to demonstrate an association between the *Bcl*I polymorphism and increased BMI values in CD patients. This study is also the first to demonstrate the possible influence of the *HSD11B1*-rs12086634 polymorphism in tumor size. These findings may provide new insights into the genetic factors that influence the phenotypic variability of CD patients. Translating these findings into clinical practice could help manage this patient group by identifying subgroups of patients who are at-risk for developing higher BMIs and associated comorbidities, such as hypertension and insulin resistance; these patients would benefit the most from personalized treatment.

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■ AUTHOR CONTRIBUTIONS

Moreira RP and Bachega TA contributed equally to this work. Bachega TA, Fragoso MC, Mendonca BB and Moreira RP conceived and designed the experiments. Moreira RP performed the experiments. Moreira RP and Bachega TA analyzed the data. Bachega TA contributed to the reagents/materials/analysis tools. Moreira RP, Bachega TA, Fragoso MC, Machado MC, Bronstein MD and Mendonca BB wrote the manuscript. Fragoso MC, Machado MC, Bronstein MD and Mendonca BB recruited and followed-up the patients.

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