BCLI POLYMORPHISM OF THE GLUCOCORTICOID RECEPTOR GENE IS ASSOCIATED WITH INCREASED OBESITY, IMPAIRED GLUCOSE TOLERANCE AND DYSLIPIDEMIA IN PATIENTS WITH ADDISON'S DISEASE

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BCLI POLYMORPHISM OF THE GLUCOCORTICOID RECEPTOR GENE IS ASSOCIATED WITH INCREASED OBESITY, IMPAIRED GLUCOSE METABOLISM AND DYSLIPIDEMIA IN PATIENTS WITH ADDISON'S DISEASE

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Abstract

Object: Although glucocorticoids are essential for health, several studies have shown that replacement glucocorticoids in Addison's disease might be involved in anthropometric and metabolic impairment, with increased cardiovascular risk, namely if conventional doses are used. As the effects of glucocorticoids are mediated by the glucocorticoid receptor, encoded by NR3C1 gene, different polymorphisms in the NR3C1 gene have been linked to altered glucocorticoid sensitivity in general population as well as in patients with obesity or metabolic syndrome. **Design**: We investigated the impact of glucocorticoid receptor gene polymorphisms, including the BclI, N363S and ER22/23EK variants, on anthropometric parameters (BMI and waist circumference), metabolic profile (HOMA, OGTT and serum lipids) and ACTH levels in 50 Addison's disease patients (34 F and 16 M, age 20-82 yr) under glucocorticoids replacement. **Results:** Neither N363S nor ER22/23EK variants were significantly associated with either anthropometric, metabolic or hormonal parameters, while patients carrying the homozygous BclI polymorphism GG (n=4) showed higher ($P<0.05$) BMI, waist circumference, HOMA and 2-h glucose levels after OGTT, as well as total cholesterol and triglycerides than those with wild-type genotype CC (n=28) or heterozygous CG (n=18). The totality of GG patients was connoted by abdominal adiposity, impaired glucose tolerance/diabetes mellitus or dyslipidemia, while a lower percentage of CC or CG patients showed some anthropometric and metabolic alterations. **Conclusion:** These results suggest that BclI polymorphism may influence the sensitivity to glucocorticoids in patients with Addison's disease and may contribute, along with other factors, to the increase of central adiposity, impaired glucose metabolism and dyslipidemia.

Introduction

In Addison's disease glucocorticoids, along with mineralocorticoids and dehydroepiandrosterone, are insufficiently secreted and GC replacement is essential for health and, indeed, life (1-3). Hydrocortisone is the preferred replacement therapy for these patients worldwide (2-4), although cortisone acetate is still the most widely used replacement therapy in Italy. Currently, glucocorticoids doses prescribed for adults, based on cortisol production rate (5) and the practicality of taking oral drugs, are generally hydrocortisone 20 mg/day or cortisone acetate 25 mg/day, while conventional doses of 30 and 37.5 mg/day of each drug are considered supra-physiological (3,4).

Once Addison's disease is diagnosed and treated, life expectancy is considered normal and without complications (6), although some studies have shown that replacement glucocorticoids treatment might be involved in anthropometric and metabolic impairment with increased cardiovascular risk, namely if conventional doses are used (3, 7-9). On the other hand, glucocorticoids under-treatment is demonstrated to be potentially life-threatening, presenting with chronic fatigue and reduced resistance to illness (10). Therefore, either over-treatment or under-treatment is hypothesized as being one of the major causes of increased mortality in this disease (7-10).

Effects of glucocorticoids treatment are known to vary considerably between patients: in fact some patients respond very well to conventional glucocorticoids doses but also develop serious side effects, while others need a very high dose to achieve clinical effect but do not suffer from side effects (3,4).

Several lines of evidence suggest that in healthy subjects as well as in some diseases (e.g obesity, dyslipidemia) the different sensitivity and individual responses to glucocorticoids are, at least partially, genetically determined (11,12). More than a dozen polymorphisms of the glucocorticoid receptor gene have been published (11,12) however, their functional and clinical impact is still largely unknown. Several genetic variations within the coding region of the glucocorticoid receptor gene, such as ER22/23EK (rs6189 and 6190) or N363S (rs6195) as well as in the intron region BclI (rs41423247), have been described and various genotype–phenotype associations have been shown (12). At least three polymorphisms seem to be associated with altered glucocorticoids sensitivity, changes in body composition and metabolic parameters. Both the BclI and the N363S polymorphisms have been shown to be associated with increased sensitivity to GC, leading to increased body mass index and insulin-resistance, whereas the ER22/23EK variant was shown to cause relative resistance to glucocorticoids, reduced body mass index and a low cardiovascular risk (12,13).

As the expression of glucocorticoid receptor is also essential for the negative feedback that closes the loop formed by corticotropin-releasing hormone, corticotropin (ACTH) and cortisol (14), baseline ACTH levels in Addison's disease patients might vary according to genetic differences of glucocorticoid receptor gene, besides the effect exerted by different

doses of glucocorticoids. On the other hand, some Authors have recently suggested that ACTH profiles could better assess the variable sensitivity to the glucocorticoids activity of cortisol in patients with Addison's disease (15).

Based on this background, we designed a clinical retrospective study aimed at assessing whether polymorphisms of the glucocorticoid receptor gene, including the BclI, N363S and ER22/23EK variants, could have an impact on anthropometric, metabolic and hormonal profiles in a group of patients with Addison's disease under glucocorticoids replacement therapy.

Subjects and methods

Fifty consecutive patients with Addison's disease diagnosed at the Division of Endocrinology, Diabetology and Metabolism, University of Turin, and at Department of Internal Medicine, University of Perugia, were studied (34 females and 16 males, age 20-82 years). Ten patients were affected by autoimmune isolated Addison's disease, 5 patients by post-tuberculosis isolated, 1 patient by autoimmune-polyendocrine syndrome type 1 while other 34 patients were affected by autoimmune-polyendocrine syndrome type 2.

The diagnosis of autoimmune Addison's disease was based on the presence of circulating adrenal autoantibodies against the steroidogenetic enzyme 21-hydroxylase (21OHAb), determined in radio binding assay that uses *in vitro* translated recombinant human ^{35}S-21OH, and expressing 21OHAb levels as a relative index (21OH index) based on the analysis of one positive and two negative standard sera included in each assay, with the upper level of normal for the 21OH index that was 0.06 (16). The diagnosis of post-tubercolosis was based on the positivity of Mantoux skin test together with specific radiological signs.

Patients with systemic autoimmune diseases under immunosuppressive steroid therapy were excluded from the study and none of the patients were receiving any drug influencing glucose or lipid metabolism. All eumenorrheic women were studied in their early follicular phase and patients with precocious ovary failure had not been in hormone replacement therapy since the year before the study. Patients with autoimmune (tyreoperoxidase antibodies >100 mU/ml) hypothyroidism were under appropriate treatment with L-thyroxin at the time of the study, leading to normal TSH, free triiodothyronine (fT3) and free thyroxine (fT4) levels.

None of the patients had experienced any cardiovascular or cerebrovascular events disease since the time of the study.

Thirty-three patients were under cortisone acetate dose (range 18.75-37.5 mg/day, twice-daily regimen) and 17 patients under hydrocortisone dose (range 15-30 mg/day, trice-daily regimen), with higher doses on awakening and lower doses in the afternoon, for a mean period of about 16 years (range 1-40 years), and under fludrocortisone treatment, at once daily doses given in the morning (0.025-0.1 mg) and which were able to normalize serum electrolytes and blood pressure. The doses of glucocorticoids were titrated on the basis of a clinical judgment in order to avoid either symptoms or classical signs of under/over-treatment.

The main demographic, anthropometric, hormonal and metabolic findings of the patients are reported in *Table 1*.

All the patients gave their informed consent to participate in the study which had been approved by the Ethical Committee of the University of Turin, in agreement with the Declaration of Helsinki.

Genetic evaluation

Total genomic DNA was isolated from 400 µl peripheral blood collected in EDTA tubes using the automatic extractor "Maxwell" (Promega). Three NR3C1 SNPs were studied by pyrosequencing assay: BclI (rs41423247:Cytosine>Guanine), N363S (rs6195:Adenine>Guanine) and ER22/23EK (rs6189:Guanine>Adenine and rs6190:Guanine>Adenine).

Polymerase chain reaction (PCR) and sequencing primers were designed using the PSQ Assay Design Software version 1.0.6 (Qiagen, Hilden, Germany). One primer of each PCR pair has a 5'-biotin modification necessary for post-PCR processing. PCR amplification for the pyrosequencing assay was performed according to standard protocols. The single-strand amplicons were mixed with sequencing primers and pyrosequencing was performed using a PyroGold reagent kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Results were analyzed using the PSQ-96MA2.0.2 software.

Clinical evaluation

Weight, height, body mass index (BMI) and waist circumference were measured by using standard methods. A BMI between 25 and 29.9 kg/m^2 was classified as overweight, a BMI of 30 kg/m^2 or more as obesity (17); waist circumference ≥ 88 cm in women and 102 cm in men were used to define abdominal adiposity (18).

Biochemical evaluation

Blood samples were taken at fast in the morning, between 0800 and 0900 h, from all patients before taking the hormonal replacement therapy. An oral glucose tolerance test (OGTT, 75 g glucose, measuring blood glucose concentrations at 0 min and 2 h) was also performed.

Serum glucose (mmol/l), total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides (mmol/l) levels were measured by enzymatic colorimetric method (Roche Diagnostic GmbH, D-68298 Mannheim, German), while insulin (pmol/l) and ACTH levels (pmol/l) were measured in duplicate by Immulite 2000 (Siemens Healthcare Diagnostics Inc., Llanberis, Gwynedd LL55 4EL, UnitedKingdom), with the sensitivity of the assay that was 14 pmol/l and 1 pmol/l, respectively and the inter-and intra-assay coefficients of variation which ranged from 4.1 to 7.3% and 6.1 to 10 %, respectively, and from 3.7 to 5.5% and 6.7 to 9.5 %, respectively.

HOMA index and low density lipoprotein (LDL) cholesterol were calculated as follows: fasting insulin (mU/l) x fasting glucose (mmol/l)/22.5 and total cholesterol - (HDL+(triglycerides/5), respectively.

According to American Diabetes Association criteria, impaired fasting glucose (IFG) was defined as fasting glucose levels ≥ 5.6 mmol/l, while impaired glucose tolerance (IGT) was diagnosed when 2h glucose levels after OGTT were between 7.8 and 11.0 mmol/l, and diabetes mellitus (DM) when glucose levels ≥ 7.0 mmol/l or 2h glucose levels after OGTT ≥ 11.1 mmol/l (19).

Hypercholesterolemia was diagnosed when total cholesterol levels were ≥6.0 mmol/l while hypertriglyceridemia when triglycerides levels were ≥ 1.7 mmol/l (20).

Statistical analysis

Quantitative data were expressed as mean ± SD and categorical data as percentage.

The statistical analysis was carried out using Mann-Whitney U test for comparison between the numerical data and by the $\chi 2$ test for the categorical data. Furthermore, the associations between genotypes and anthropometric/biochemical/hormonal parameters were evaluated using Kruskal–Wallis ANOVA. Post hoc comparison between groups was performed using the MannWhitney test, with Bonferroni correction of significance level.

Analysis of covariance (ANCOVA) was used to assess the association between anthropometric/biochemical/hormonal parameters, using genotypes as a factor while sex, age, disease duration and daily GC dose as covariates. Hardy–Weinberg equilibrium for BclI variants was calculated.

All statistical analyses were performed using Statistical Package for the Social Science (SPSS 19.0 for Windows: SPSS Inc., 1989-2005, Chicago IL, USA).

A value of $P<0.05$ was considered to be significant.

Results

Allelic frequencies of the GR gene polymorphisms

The BclI polymorphism was in Hardy-Weinberg equilibrium, while the Hardy-Weinberg equilibrium was not calculated for the N363S and ER22/23EK polymorphisms because of the low frequency of the polymorphic genotypes. Genotype distribution and allele frequencies of the BclI GR gene polymorphism are reported in *Table 2*.

GR genotypes in relation to anthropometric, metabolic and hormonal findings

Neither N363S nor ER22/23EK variants were significantly associated with any anthropometric, metabolic or hormonal parameters, while the BclI genotype showed a significant association ($P<0.05$) with BMI, waist circumference, HOMA, 2-h glucose levels, total cholesterol, triglycerides and ACTH levels. No significant association was found between BclI genotype and disease duration or daily glucocorticoids dose.

No significant association between BMI, waist or the gluco-lipid parameters and ACTH levels, disease duration or daily glucocorticoids dose was found.

Patients carrying the homozygous BclI polymorphism Guanine-Guanine (GG; n=4) showed higher (P<0.05) BMI (28.4±1.9 Kg/m^2) and waist circumference (103.0±11.5 cm) than those with wild-type genotype Cytosine-Cytosine (CC; n=28; 24.8±4.2 Kg/m^2 and 88.7±11.7 cm, respectively) or heterozygous Cytosine-Guanine (CG; n=18; 24.0±2.6 Kg/m^2 and 84.1±12.2 cm), while no differences were found between CC and CG. The totality of GG patients were obese (n=1) or overweight (n=3) and connoted by abdominal adiposity, compared with a lower percentage of CC (3 obese, 9 overweight, 39% with abdominal adiposity) or CG patients (7 overweight, 28% with abdominal adiposity) *(Tab. 1 and 3, Fig. 1*) .

Mean fasting glucose and insulin showed a trend towards an increase in Bcl1 GG genotype group, while HOMA and 2h glucose levels after OGTT were significantly higher (P<0.05) in GG (2.8±0.8 and 9.5±4.0 mmol/l, respectively) than in CC (1.8±1.5 and 6.7±2.6 mmol/l, respectively) or CG (1.6±1.2 and 6.2±2.6 mmol/l, respectively). A higher percentage of GG patients (50%) showed IGT or DM, while no more than 18% of CC and 11% of CG showed any glucometabolic impairment (*Tab. 1 and 3, Fig. 2*).

Mean fasting total cholesterol and triglycerides were higher (P<0.05) in GG (5.9±0.7 and 2.3±1.5 mmol/l, respectively) than in CC (5.0±0.8 and 1.5±0.9 mmol/ll, respectively) or CG (5.2±0.9 and 1.1±0.6 mmol/ll, respectively). A high percentage of GG patients showed hypercholesterolemia (50%) or hypertryglyceridemia (75%), while no more than 11 and 25 % of CC (3 and 7 out of 28) and 22 and 6% of CG (4 and 1 out of 18) showed any lipidic alterations (*Tab. 1 and 3, Fig. 2*).

ACTH levels were lower (P<0.05) in patients carrying the homozygous BclI polymorphism GG (53.0±33.0 pmol/l) than those with wild-type genotype CC (134.0±45.0 pmol/l), or heterozygous CG (82.0±34.0 pmol/l), while no significant differences were found between CC and CG (*Tab. 1 and 3*).

Discussion

In the present study, we investigated the frequencies and the potential involvement of three functionally important polymorphisms of the glucocorticoid receptor gene (BclI, N363S and ER22/23EK) in the pathogenesis of central adiposity, impaired glucose tolerance and dyslipidemia in patients with Addison's disease under glucocorticoids replacement therapy.

We found that the allele frequencies of these polymorphisms were similar to those reported in several previous studies involving healthy subjects (12), suggesting that these polymorphisms do not modify the susceptibility to Addison's disease. Accordingly, inclusion of healthy control samples was not deemed relevant for the objective of the study which was to evaluate the role of glucocorticoid receptor gene polymorphisms in modulating the anthropometric and metabolic profile in patients with Addison's disease.

However, our present results show that patients with Addison's disease carrying the polymorphic BclI allele in a homozygous variant had a higher prevalence of central adiposity, impaired glucose tolerance, diabetes mellitus and dyslipidemia, compared to patients with the wild-type BclI variant. The lack of difference in these clinical parameters between the CG and the wild-type (CC) genotypes suggests that the BclI variant exerts its effect primarily in the homozygous polymorphic form, in agreement with other reports although in a different disease (21). However, further functional investigations are needed to clarify this issue.

Individual sensitivity to glucocorticoids is determined by diverse mechanisms including metabolism of glucocorticoids by 11β-hydroxysteroid dehydrogenases, the glucocorticoid receptor number and affinity, and interactions of the glucocorticoid receptor with various nuclear cofactors (14). Several lines of evidence suggest that the different sensitivity and individual responses to glucocorticoids are at least partially genetically determined (12,13). A number of studies have investigated the impact of the glucocorticoid receptor gene polymorphisms on glucocorticoids sensitivity, that may exhibit a tissue-specific effect (11, 22), namely in healthy subjects.

At least three polymorphisms seem to be associated with altered lucocorticoids sensitivity and changes in body composition and metabolic parameters.

For the BclI polymorphism, a relative glucocorticoids sensitivity has been demonstrated and this genotype has been shown to be associated with increased abdominal obesity and insulin-resistance in the general population (12, 13, 22-24), while other studies found no association with either obesity or cardiovascular disease risk factors, such as total and LDL-cholesterol, triglycerides, and HbA1c (24,25).

Similarly, the N363S polymorphism was reported as being associated with an enhanced sensitivity to glucocorticoids, with increased body mass index (12,13,22, 26), waist to hip ratio (27) and LDL-cholesterol levels, as well as with increased risk of cardiovascular disease (26), although conflicting data are present for this variant (28).

In contrast, the ER22/23EK polymorphism was associated with a relative glucocorticoids resistance, with a favorable metabolic profile with lower total and LDL-cholesterol levels (29), a better insulin sensitivity and favorable body compositional conditions (30). In elderly subjects this polymorphism was associated with longevity (31).

Differently form healthy subjects, only few studies have been conducted in patients with alteration of cortisol secretion such as endogenous hypercortisolism. In particular, some Authors have reported an association between BclI polymorphism and bone mineral density (21). Although it might be useful to screen for the presence of these glucocorticoid receptor gene variants in order to determine an individual's dose of glucocorticoids, to our knowledge, no studies in patients with primary hypoadrenalism do exist. Few reports performed in patients with congenital adrenal hyperplasia, showed that females carriers of the N363S polymorphism were characterized by milder genital virilization at birth than non-carriers (32),

possibly reflecting a compensatory reduction in ACTH production as a result of increased glucocorticoids sensitivity. No significant differences in substitution doses, hormonal or auxologic parameters between carriers and non-carriers were observed.

Concerning anthropometric parameters, a higher BMI and waist circumference as well as a higher percentage of central fat distribution was present in our patients with homozygous GG variant. However, no association between BMI, waist circumference, disease duration or daily glucocorticoids doses was recorded in the whole group of patients, thus suggesting a lacking influence of either the duration of the exposure to glucocorticoids or the dose of glucocorticoids on this anthropometric parameter. These data are at variance with those from other studies conducted in secondary hypoadrenalism, which showed a direct correlation between GC doses and visceral adiposity (33). Unfortunately, we did not performed a complete body composition evaluation that would have better differentiated our patients.

A derangement of glucose metabolism and insulin sensitivity, in terms of higher HOMA and impaired glucose response to OGTT, was present in a higher percentage of homozygous GG patients than in wild-type or heterozygous variants, while no differences were found in both fasting glucose and insulin levels. Once again, no association between these glycaemic parameters and disease duration or daily glucocorticoids doses was reported, thus suggesting that the influence of long-term glucocorticoids replacement therapy on glucose metabolism is less than expected, differently from what was reported, namely in secondary hyoadrenalism (34, 35).

As far as the lipid profile is concerned, we found a higher prevalence of hypercholesterolemia and hypertriglyceridemia in homozygous GG patients, than in wild-type or heterozygous variants. The lacking correlation between lipid profile and either disease duration or daily glucocorticoids doses recorded in our study does not support the influence of chronic glucocorticoids replacement therapy on the occurrence of dyslipidemia in Addison's disease.

Finally, we found that ACTH levels were lower in homozygous GG patients than in wild-type or heterozygous variants, independently of the glucocorticoids dose as well as disease duration. From a clinical point of view this result seems to be important. In fact, differently from the general opinion that in Addison's disease patients ACTH levels before the morning glucocorticoids dose are invariably high and they rapidly decline with increasing cortisol levels after glucocorticoids ingestion (1-4), our findings suggest that the lower ACTH levels observed in patients with homozygous GG may reflect a more efficient negative feedback exerted by glucocorticoids to corticotropic cells (14), in agreement with the higher sensitivity to glucocorticoids showed in subjects carrying this polymorphism.

Our study shows some limitations. The major limit was the small number of patients studied, which does not allow us to drawn any definite conclusions about the relationship between glucocorticoids polymorphisms and clinical features in patients with Addison's disease, and our findings might have come about just by chance. However, Addison's disease is a

rare disorder with an estimated annual incidence of 4.7-6.2 million/year (2), thus, although the number of patients in our study was relatively small, the number was sufficient to detect significant findings not previously reported as to the potential role of this glucocorticoid receptor polymorphism on various parameters. Other limits were the heterogeneity of patients studied (disease's duration, glucocorticoids type and dose), which do not allow us to state definite conclusions about the morbidity, and the fact that we were unable to directly correlate the exposure to replacement GC with the metabolic profile, as we neither performed serum cortisol day curves nor managed a monitoring of glucocorticoids replacement. However, we emphasize that no definite conclusions can be drawn in the case of the two polymorphisms N363S and ER22/23EK with rather low frequencies.

Despite these limitations, the strength of our study is the novelty of the observation concerning the association between Bcl1 polymorphism and central adiposity, impaired glucose tolerance and dyslipidemia in patients with Addison's disease, as well as, from the clinical point of view, the importance of evaluating these glucocorticoid receptor gene variants in order to determine an individual dose of glucocorticoids.

In conclusion, the results of our study, although preliminary, suggest that the BclI polymorphism may influence the sensitivity to glucocorticoids in patients with Addison's disease and may contribute, along with other factors, to the increase of central adiposity, impaired glucose metabolism and dyslipidemia, although further studies in larger cohorts of patients with primary adrenal insufficiency should be performed to draw definite conclusions.

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Legend to Tables

Table 1

CC: Cytosine-Cytosine; CG: Cytosine-Guanine; GG: Guanine-Guanine; AA: Adenine-Adenine; AG: Adenine-Guanine; GA: Guanine-Adenine. Results are presented as mean±SD.

Table 2

CC: Cytosine-Cytosine; CG: Cytosine-Guanine; GG: Guanine-Guanine; AA: Adenine-Adenine; AG: Adenine-Guanine; GA: Guanine-Adenine.

Table 3

CC: Cytosine-Cytosine; CG: Cytosine-Guanine; GG: Guanine-Guanine; AA: Adenine-Adenine; AG: Adenine-Guanine; GA: Guanine-Adenine; IGT: impaired glucose tolerance; DM: diabetes mellitus. Results are presented as mean±SD.

Legend to Figures

Fig. 1 Percentage of patients (%) with central adiposity (waist > 88 or 102 cm in women and men, respectively) in patients with Addison's disease of different genotypes (CC: Cytosine-Cytosine; CG: Cytosine-Guanine; GG: Guanine-Guanine; AA: Adenine-Adenine; AG: Adenine-Guanine; GA: Guanine-Adenine)

Fig. 2 Percentage of patients (%) with impaired glucose tolerance (IGT) or diabetes mellitus (DM) or total cholesterol > 240 mg/dl or triglycerides > 150 mg/dl in patients with Addison's disease of different genotypes (CC: Cytosine-Cytosine; CG: Cytosine-Guanine; GG: Guanine-Guanine; AA: Adenine-Adenine; AG: Adenine-Guanine; GA: Guanine-Adenine)

Table 1. Demographic, clinical, anthropometric, hormonal and metabolic details in patients with Addison's disease.

		BclI Allelic variants		
		CC	**CG**	**GG**
Numbers	50	28	18	4
Female/male	34/16	17/11	13/5	4/0
Mean age (yr)	51.3±14.0	47.9±2.6	54.1±3.6	60.5±4.0
Glucocorticoids mean daily dose (mg)				
cortisone acetate (n=33)	30.2±6.2	(n=19) 31.6±6.4	(n=11) 30.1±5.5	(n=3) 27.1±9.
hydrocortisone (n=17)	21.9±4.5	(n=9) 21.4±5.2	(n=7) 21.7±4.1	(n=1) 25.0±-
Mean duration (yr)	16.6±12.1	16.2±12.6	16.2±11.3	16.5±20.5
Mean BMI (Kg/m^2)	24.8±3.6	24.8±4.2	24.0±2.6	28.4±1.9
Mean waist (cm)				
F (n=34)	86.5±12.3	(n=17) 86.8±9.6	(n=13) 80.9±11.6	(n=4) 103.0±11
M (n=16)	91.8±13.0	(n=11) 91.5±14.5	(n=5) 92.2±10.5	(n=0) -
Mean glucose (mmol/l)	4.8±1.2	4.7±0.6	4.5±0.5	6.6±3.4
Mean insulin (pmol/l)	60.0±13.1	60.0±37.5	53.0±36.7	85.0±36.4
Mean HOMA	1.8±0.8	1.8±1.5	1.6±1.2	2.8±0.8
Mean 2h-glucose after OGTT (mmol/l)	6.7±1.4	6.7±2.6	6.2±2.6	9.5±4.0
Mean total cholesterol (mmol/l)	5.0±1.1	5.0±0.8	5.2±0.9	5.9±0.7
Mean HDL-cholesterol (mmol/l)	2.0±0.4	1.0±0.5	2.0±0.5	2.0±0.9
Mean LDL-cholesterol (mmol/l)	3.0±0.8	3.0±0.8	3.0±0.8	4.0±0.3
Mean triglycerides (mmol/l)	1.4±0.9	1.5±0.9	1.1±0.6	2.3±1.5
Mean ACTH (pmol/l)	119.0±40.0	134.0±45.0	82.0±34.0	53.0±33.0

Table 2. Genotype distribution and allele frequencies of the glucocorticoid receptor gene polymorphism in patients with Addison's disease.

	Genotype distribution	**Polymorphic allele frequency**
BclI		
CC	28 (56%)	
CG	18 (36%)	0.26
GG	4 (8%)	
N363S		
AA	49 (98%)	
AG	1 (2%)	0.01
GG	0	
ER22/23EK		
GG	49 (98%)	
GA	1 (2%)	0.01
AA	0	

Table 3. Demographic, clinical, anthropometric, hormonal and metabolic parameters according to BclI genotypes in patients with AD.

	BclI Allelic variants				
	CC	**CG**	**GG**	**P (GG vs CC)**	**P (GG vs CG)**
Numbers	28	18	4		
Female/male	17/11	13/5	4/0		
Mean age (yr)	47.9±2.6	54.1±3.6	60.5±4.0	0.11	0.11
Glucocorticoids mean daily dose (mg)					
cortisone acetate	(n=19) 31.6±6.4	(n=11) 30.1±5.5	(n=3) 27.1±9.5	0.60	0.50
hydrocortisone	(n=9) 21.4±5.2	(n=7) 21.7±4.1	(n=1) 25±-	0.90	0.90
Mean duration (yr)	16.2±12.6	16.2±11.3	16.5±20.5	0.70	0.70
Mean BMI (Kg/m^2)	24.8±4.2	24.0±2.6	28.4±1.9	0.048	0.048
Obese (n)	3	0	1		
Overweight (n)	9	7	3		
Mean waist (cm)	88.7±11.7	84.1±12.2	103.0±11.5	0.049	0.047
Waist≥88 or 100 cm (n)	11	5	4		
Mean glucose (mmol/l)	4.7±0.6	4.5±0.5	6.6±3.4	0.10	0.70
Mean insulin (pmol/l)	60.0±37.5	53.0±36.7	85.0±36.4	0.70	0.50
Mean HOMA	1.8±1.5	1.6±1.2	2.8±0.8	0.047	0.046
Mean 2h-glucose after OGTT (mmol/l)	6.7±2.6	6.2±2.6	9.5±4.0	0.047	0.045
IGT	4	1	1		
DM	1	1	1		
Mean total cholesterol (mmol/l)	5.0±0.8	5.2±0.9	5.9±0.7	0.046	0.048
Mean HDL-cholesterol (mmol/l)	1.0±0.5	2.0±0.5	2.0±0.9	0.080	0.080.
Mean LDL-cholesterol (mmol/l)	3.0±0.8	3.0±0.8	4.0±0.3	0.265	0.265
Hypercholesterolemic (n)	3	4	2		
Mean triglycerides (mmol/l)	1.5±0.9	1.1±0.6	2.3±1.5	0.049	0.046
Hypertryglyceridemic (n)	7	1	3		
Mean ACTH (pmol/l)	134.0±45.0	82.0±34.0	53.0±33.0	0.020	0.040