A Polymorphism in the Glucocorticoid Receptor Gene, Which Decreases Sensitivity to Glucocorticoids In Vivo, Is Associated With Low Insulin and Cholesterol Levels

Elisabeth F.C. van Rossum,[1] Jan W. Koper,[1] Nannette A.T.M. Huizenga,[1] André G. Uitterlinden,[1] Joop A.M.J.L. Janssen,[1] Albert O. Brinkmann,[2] Diederick E. Grobbee,[3] Frank H. de Jong,[1] Cornelia M. van Duyn,[4] Huibert A.P. Pols,[1] and Steven W.J. Lamberts[1]

We investigated whether a polymorphism in codons 22 and 23 of the glucocorticoid (GC) receptor gene [GAGAGG(GluArg) → GAAAAG(GluLys)] is associated with altered GC sensitivity, anthropometric parameters, cardiovascular risk factors, and sex steroid hormones. In a subgroup of 202 healthy elderly subjects of the Rotterdam Study, we identified 18 heterozygotes (8.9%) for the 22/23EK allele (ER22/23EK carriers). In the highest age group, the number of ER22/23EK carriers was higher (67–82 years, 12.9%) than in the youngest age group (53–67 years, 4.9%; $P < 0.05$). Two dexamethasone (DEX) suppression tests with 1 and 0.25 mg DEX were performed, and serum cortisol and insulin concentrations were compared between ER22/23EK carriers and noncarriers. After administration of 1 mg DEX, the ER22/23EK group had higher serum cortisol concentrations (54.8 ± 18.3 vs. 26.4 ± 1.4 nmol/l, $P < 0.0001$), as well as a smaller decrease in cortisol (467.0 ± 31.7 vs. 484.5 ± 10.3 nmol/l, $P < 0.0001$). ER22/23EK carriers had lower fasting insulin concentrations ($P < 0.001$), homeostasis model assessment–insulin resistance (IR) (index of IR, $P < 0.05$), and total ($P < 0.02$) and LDL cholesterol concentrations ($P < 0.01$). Our data suggest that carriers of the 22/23EK allele are relatively more resistant to the effects of GCs with respect to the sensitivity of the adrenal feedback mechanism than noncarriers, resulting in a better metabolic health profile. *Diabetes* 51:3128–3134, 2002

From the [1]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands; the [2]Department of Endocrinology & Reproduction, Erasmus Medical Center, Rotterdam, the Netherlands; the [3]Department of Julius Center, University of Utrecht, Utrecht, the Netherlands; and the [4]Department of Epidemiology & Biostatistics, Erasmus Medical Center, Rotterdam, the Netherlands.

Address correspondence and reprint requests to J.W. Koper, Department of Internal Medicine, Room Bd 277, Erasmus Medical Center, P.O. Box 2040, 3000 CA Rotterdam, the Netherlands. E-mail: koper@inw3.fgg.eur.nl.

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CBG, cortisol-binding globulin; DEX, dexamethasone; DST, DEX suppression test; DHEAS, dehydroepiandrosterone-sulfate; GC, glucocorticoid; GR, GC receptor; HOMA, homeostasis model assessment; HOMA-B, HOMA β-cell function; HPA, hypothalamo-pituitary-adrenal; IGF-BP1, IGF-binding protein 1; IR, insulin resistance; RFLP, restriction fragment–length polymorphism; RIA, radioimmunoassay; SHBG, sex hormone–binding globulin.

Glucocorticoids (GCs) are important regulators in almost every tissue in the human body, and their effects are mediated by the GC receptor (GR) (1). A complete inability of GCs to exert their effects on target tissues is probably not compatible with life. However, several patients have been described with partial forms of GC resistance. They show a wide spectrum of clinical symptoms, such as hypertension, hypokalemic alkalosis (2), fatigue, and hyperandrogenism (3). Vingerhoeds et al. (2) reported a father and a son with GC resistance, and from then until now 20 additional patients and family members with this syndrome have been described (4).

Besides these symptomatic patients with relative GC resistance, within the normal population, a considerable variability in the feedback sensitivity of the hypothalamo-pituitary-adrenal (HPA) axis was also demonstrated (5). The molecular mechanisms underlying this variation in GC sensitivity are still largely unknown. In the symptomatic patients with familial forms of GC resistance, missense mutations in the ligand binding domain of the GR gene, causing decreased ligand binding affinity, have been described (6,7), as well as a deletion of four base pairs at the boundary of exon 6 and intron 6, causing loss of a splice site and a 50% reduction of receptor numbers per cell, resulting also in GC resistance (8). Within the normal population, several polymorphisms in the GR gene have been reported (9). One of these polymorphisms consists of a point mutation in codon 363 in exon 2 of the GR gene, resulting in an asparagine-to-serine amino acid change, and was shown to be associated with an increased sensitivity to GCs in response to dexamethasone (DEX) (10). Another polymorphism consists of two linked point mutations separated by one base pair in codons 22 and 23 in exon 2 of the GR gene. The mutations are located at cDNA positions 198 and 200, respectively. The first mutation is silent, changing codon 22 from GAG to GAA, both coding for glutamic acid (E). The second mutation changes codon 23 from AGG to AAG, resulting in an amino acid change from arginine (R) to lysine (K) (9). These mutations did not seem to alter the activity of the GR during in vitro experiments (11). However, the clinical relevance of this polymorphism has not been studied. Within the context of an ongoing population-based cohort study of diseases in

the elderly (the Rotterdam Study), we investigated whether there were any differences in vivo between ER22/23EK carriers and noncarriers in the sensitivity of the HPA axis to the overnight administration of 1 or 0.25 mg DEX, as well as in some anthropometric parameters, cardiovascular risk factors, and sex steroid hormone levels.

RESEARCH DESIGN AND METHODS

A total of 202 individuals participated in the study. Their age varied between 53 and 82 years (98 men and 104 women, with mean ages of 67.7 ± 0.6 and 65.9 ± 0.6 years, respectively). They were living in a suburb of Rotterdam, the Netherlands. These subjects were participants in the Rotterdam Study, a population-based cohort study (7,983 subjects) of the determinants of chronic disabling diseases in the elderly, and they were selected at random. Subjects with acute psychiatric or endocrine diseases, including diabetes treated with medication, were not invited. Compared with all participants of the Rotterdam study, there were no differences in age and sex distribution and cardiovascular risk factors. The subjects gave their written consent to participate in the study, which received the approval of the Medical Ethics Committee of the Erasmus University Medical School. To get more information about the individual variability of the feedback sensitivity of the HPA axis, all 202 subjects were invited for a second DEX suppression test (DST) with a lower-dose DEX (0.25 mg) 2.5 years later. A total of 149 subjects agreed to participate in this second test (72 men and 77 women).

Anthropometric measurements The body weight, height, and waist-to-hip ratio of the subjects were measured, and the BMI (in kg/m^2) was calculated. Blood pressure was measured in the sitting position at the right upper arm with a random-zero sphygmomanometer.

DSTs The two DSTs were performed as described previously (5). In brief, venous blood was obtained between 8:00 and 9:00 A.M., after an overnight fast, for serum cortisol and insulin measurements. Participants were instructed to ingest a tablet of 1 mg (and 0.25 mg for the second DST) DEX at 11:00 P.M.. The next morning, fasting blood was drawn by venapuncture at the same time as the previous morning. To check for compliance and possible abnormalities in the metabolism of DEX, the DEX concentration was also measured in a radioimmunoassay (RIA), using antiserum obtained from IgG Corporation (Nashville, TN). Intra- and interassay variations were below 8.5 and 14.2%, respectively.

Hormonal measurements Serum cortisol concentrations were determined using RIA kits obtained from Diagnostics Products (Los Angeles, CA). Intra- and interassay variations were below 8.0% and 9.5%, respectively. Circulating insulin and cortisol-binding globulin (CBG) concentrations were determined using commercially available RIAs (Medgenix Diagnostics, Brussels, Belgium). Intra- and interassay variations were 8.0 and 13.7%, respectively. Estradiol, androstenedione, and dehydroepiandrosterone-sulfate (DHEAS) concentrations were determined using RIA kits obtained from Diagnostics Products. Intra- and interassay variations, respectively, were as follows: estradiol 7.0 and 8.1%, androstenedione 8.3 and 9.2%, and DHEAS 5.3 and 7.0%. Sex hormone–binding globulin (SHBG) was assayed with a commercially available immunoradiometric assay (Diagnostics Products); intra- and interassay variations were 3.6 and 6.9%, respectively. Testosterone was measured with a noncommercial RIA (intra- and interassay variations were 3.6 and 6.9%). Commercially available immunoradiometric assays were used for the measurement of IGF-binding protein 1 (IGF-BP1; Diagnostic System Laboratories); intra- and interassay variations were 4.0 and 6.0%. Insulin resistance (IR) and β-cell function were estimated using the homeostasis model assessment (HOMA), using the following previously described formula: (fasting insulin [in mU/l] × fasting glucose [in mmol/l])/22.5 (12,13).

Biochemical measurements Glucose, total cholesterol, HDL cholesterol, and triglycerides were measured using standard laboratory methods. LDL cholesterol was calculated using the following formula: LDL cholesterol = total cholesterol − ([triglycerides/5] + HDL cholesterol).

Genetic analysis Restriction fragment–length polymorphism (RFLP) analysis was carried out to determine GR genotypes. DNA was extracted from peripheral blood leukocytes using standard techniques. PCR amplification of the GR gene was carried out using primer sequences and amplification conditions, as described previously (9). The PCR products were digested with 1 unit Mnl I (New England Biolabs) at 37°C for 1 h. Mnl I cleaves at 5′-CCTC(N)7-′3 and at 3′-GGAG(N)6-′5. Fragments were visualized with ethidium bromide on a 3% agarose gel (MP-Boehringer, Mannheim, Germany). We reanalyzed the 18 heterozygous and 10 wild-type samples and found identical genotypes.

Statistical analysis Data were analyzed using SPSS for Windows, release 9.0 (SPSS, Chicago, IL). Logarithmic transformations were applied to normalize variables and to minimize the influence of outliers. Differences between the ER22/23EK carriers and the noncarriers were adjusted for age and sex and tested by ANCOVA using the general linear model procedure. A paired-samples t test was used to compare changes in insulin concentrations before and after the administration of DEX in all subjects. Results are reported as means ± SE.

Pearson's correlation coefficients were used to calculate correlations between cortisol, insulin, and cholesterol after correction for age, sex, and, if necessary, BMI. The two age groups were chosen based on the median age (67.02 years). Comparison of the frequencies of the genotypes between different age groups was carried out using a χ^2 test. P values are two-sided throughout, and $P < 0.05$ was considered to indicate a significant difference.

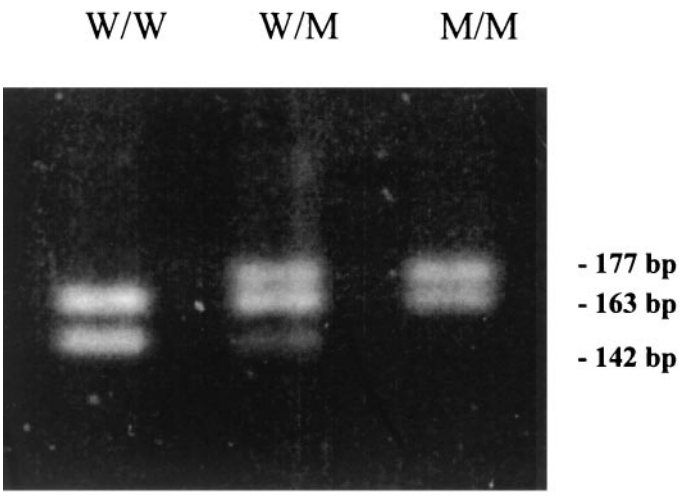

FIG. 1. Representative electrophoretic pattern of the GR22/23 genotype analyzed by PCR-RFLP from three subjects with the wild-type ER22/23ER (WW, *lane 1*), heterozygous ER22/23EK (WM, *lane 2*), and homozygous EK22/23EK (MM, *lane 3*) genotypes. ER22/23ER yielded a 163- and a 142-bp fragment; ER22/23EK appeared as three fragments of 177, 163, and 142 bp; whereas EK22/23EK consisted of a 177- and 163-bp fragment. (The homozygously affected individual was discovered in a related population study in young children. Preliminary investigations did not reveal specific anthropometric phenotypic changes in this individual).

RESULTS

RFLP analysis revealed in the study population of 202 subjects a total of 18 individuals (8.9%) who were heterozygous for the polymorphism in codon 22/23 (see also Figs. 1 and 2). No individuals homozygous for this polymorphism were found in this group. The allele frequency of the variant allele in this group was 4.5%. Genotype distributions did not differ from those expected under Hardy-Weinberg equilibrium conditions; however, we cannot say this with absolute certainty, because we did not find any homozygous ER22/23EK carriers. Sexes were equally represented in the group of ER22/23EK carriers (9 men and 9 women), as well as in the group of noncarriers (89 men and 95 women). The ER22/23EK carriers were 2.7 years older than noncarriers, and this difference did not reach statistical significance ($P = 0.09$) (Table 1). However, in the age-group between 67 and 82 years ($n = 101$), the number of ER22/23EK carriers was higher (12.9%) than in the age-group between 53 and 67 years ($n = 101$, 4.9% ER22/23EK carriers; $P < 0.05$). To rule out the influences of differences in age, all parameters were adjusted for age. No significant differences in anthropometric parameters or blood pressure between the groups were present, as shown in Table 1. At the second examination after 2.5 years, 149 of the initial 202 individuals participated (74%),

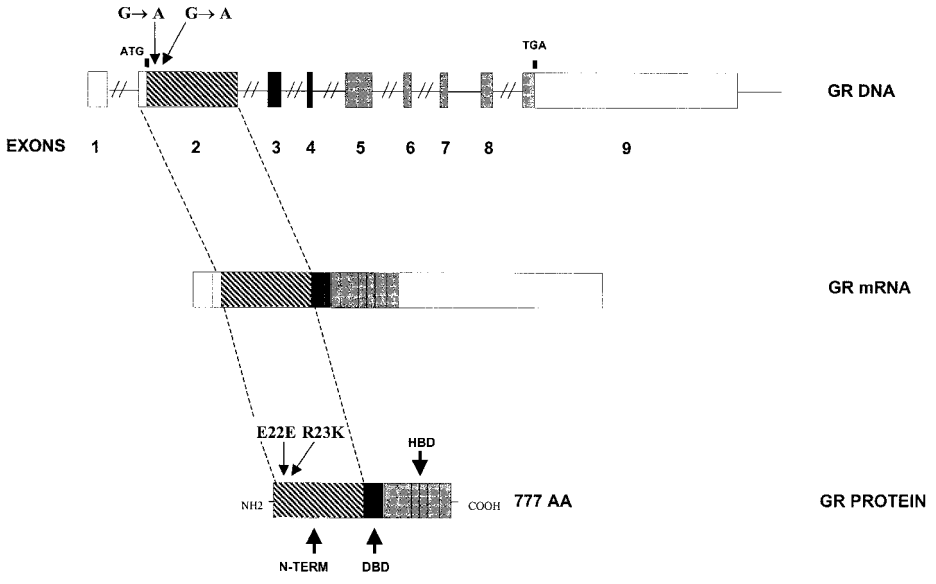

FIG. 2. Structure of the human GR gene, mRNA, and protein and its functional domains. The position of an arginine-to-lysine change at codon 23, which results from the G-to-A point mutation, and the silent G-to-A point mutation at codon 22 are indicated. N-TERM, NH_2-terminal domain; DBD, DNA binding domain; HBD, hormone binding domain.

13 of whom were heterozygous for the codon 22/23 polymorphism. Also, in this group of ER22/23EK carriers, the sexes were equally represented (six men and seven women). The group of noncarriers now consisted of 66 men and 70 women.

Feedback sensitivity of the HPA axis. Table 2 shows the concentrations of early morning serum cortisol concentrations before and after administration of 1 mg DEX, the DEX concentration, and the cortisol suppression in reaction to DEX (Δ cortisol). Three subjects were taking estrogen-containing medication, and because of the significant effect on CBG and, therefore, cortisol concentration, they were excluded from the analysis (one of the ER22/23EK carriers and two of the noncarriers) (14). One male subject had not taken the 1 mg DEX tablet and was excluded as well. There were no differences between the noncarriers and the ER22/23EK carriers in fasting cortisol concentrations. However, the cortisol concentrations after the 1-mg DST were significantly higher in ER22/23EK carriers than in noncarriers (54.8 ± 18.3 and 26.4 ± 1.4 nmol/l in ER22/23EK carriers and noncarriers, respectively; $P < 0.0001$). The absolute decrease of serum cortisol concentrations after DEX, as well as the ratio of post-DST cortisol to fasting cortisol, were significantly different (Δ cortisol: 467.0 ± 31.7 nmol/l in ER22/23EK carriers and 484.5 ± 10.3 nmol/l in noncarriers, $P < 0.0001$; ratio post-DEX to pre-DEX cortisol: 0.111 ± 0.04 nmol/l in ER22/23EK-carriers and 0.054 ± 0.00 nmol/l in noncarriers, $P = 0.003$). The significant difference in post-DEX cortisol concentrations was still present after inclusion of the three estrogen-taking subjects. The actual DEX concentrations did not differ in both groups, so the higher post-DEX cortisol levels and the smaller change in cortisol after DEX in the ER22/23EK carriers were not caused by differences in the metabolism of DEX. Also, fasting CBG levels were not different between ER22/23EK carriers and noncarriers (data not shown).

Also shown in Table 2 are the same parameters before and after the administration of 0.25 mg DEX. Again, there were no significant differences in fasting cortisol. The post-DEX cortisol concentrations and the decrease in cortisol concentrations after the administration of 0.25 mg DEX, as well as the ratio of post-DEX to pre-DEX cortisol, were not significantly different between ER22/23EK carriers and noncarriers.

Insulin and glucose concentrations. Figure 3*A* shows the fasting insulin concentrations before and after the administration of 1 and 0.25 mg DEX, respectively. To be certain that only the data from subjects with normal carbohydrate tolerance were analyzed, subjects who had developed either hyperinsulinemia or diabetes after inclusion in the study (fasting insulin values >25 mU/l or glucose concentrations of >7.8 mmol/l) were excluded from this analysis (17 noncarriers excluded, $n = 167$; 3

TABLE 1
Anthropometric parameters and blood pressure in noncarriers ($n = 184$) and ER22/23EK carriers ($n = 18$) at baseline

	Noncarriers			ER22/23EK carriers			
	Mean	SE	Range	Mean	SE	Range	*P*
Age (years)	66.5	0.44	53.0–81.6	69.2	1.68	53.5–82.4	0.09
Height (cm)	17.0	0.01	146–189	16.9	0.02	152–175	0.85
Weight (kg)	74.7	1.15	45.8–121.0	71.9	1.97	43–89.9	0.69
BMI (kg/m^2)	26.4	0.28	16.4–43.1	25.4	0.85	16.4–32.5	0.25
WHR	0.92	0.01	0.66–1.12	0.94	0.02	0.75–1.08	0.62
SBP (mmHg)	138.9	1.42	99–185	140.2	5.03	96–178	0.86
DBP (mmHg)	74.7	0.73	48–97	77.1	2.95	44–97	0.42

Test for the difference between noncarriers and ER22/23EK carriers. All parameters were log transformed and, with the exception of age, adjusted for age. DBP, diastolic blood pressure; SBP, systolic blood pressure; WHR, waist-to-hip ratio.

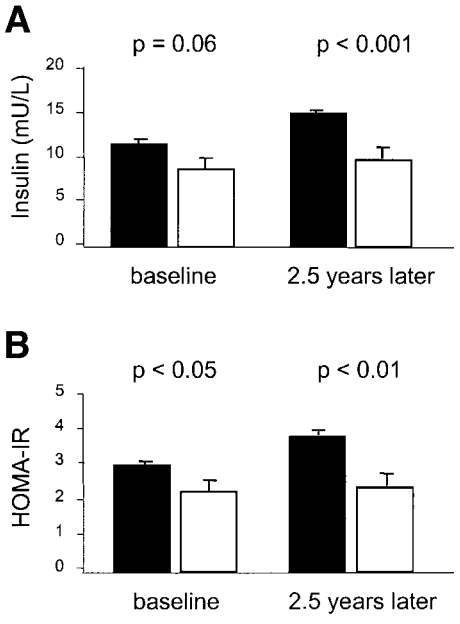

FIG. 3. *A*: Fasting insulin concentrations in noncarriers (n = 167) and ER22/23EK carriers (n = 15) before 1 mg DEX at first examination. Insulin concentrations tended to be lower in ER22/23EK carriers than noncarriers (P = 0.06). The right column shows fasting insulin concentrations in noncarriers (n = 105) and ER22/23EK carriers (n = 10) at second examination (2.5 years later). Fasting insulin concentrations were significantly lower in ER22/23EK carriers (P < 0.001). *B*: HOMA-IR scores at baseline and at second examination 2.5 years later in noncarriers and ER22/23EK carriers. At both measurements ER22/23EK carriers were significantly less insulin resistant. All parameters were log transformed and adjusted for age. Subjects with fasting insulin >25 mU/l or fasting glucose >7.8 mmol/l were excluded from the calculation; 3 ER22/23EK carriers and 17 noncarriers were excluded from the first test and 3 ER22/23EK carriers and 31 noncarriers were excluded from the second test. ■, noncarriers; □, ER22/23EK carriers

ER22/23EK carriers excluded, n = 15 at the first examination). In these 182 subjects together, a significant increase in insulin concentrations in response to the administration of 1 mg DEX was noted (11.5 ± 5.15 mU/l before and 17.2 ± 8.41 mU/l after DEX administration, respectively; $P < 0.001$). There were no differences in this increase in serum insulin concentrations between the control group and the ER22/23EK carriers (5.7 ± 0.6 vs. 5.5 ± 1.3 mU/l).

The fasting insulin concentrations tended to be lower in ER22/23EK carriers than in noncarriers (P = 0.06) (Fig. 3*A*). The same applied to the fasting serum insulin levels measured after 1 mg DEX (P = 0.07). These differences in post-DEX insulin concentrations were not caused by differences in DEX concentrations between the two groups. Fasting glucose concentrations were not different between the noncarriers and ER22/23EK carriers (5.71 ± 0.05 vs. 5.69 ± 0.16 mmol/l, respectively).

At second examination, 2.5 years later, the fasting insulin levels in ER22/23EK carriers were significantly lower than in the noncarriers ($P < 0.001$). Insulin levels decreased in the total group of 115 subjects after the administration of 0.25 mg DEX (14.7 ± 0.45 before and 13.9 ± 0.50 mU/l after DEX administration, respectively; $P < 0.01$). There were no differences in this decrease in insulin levels between the noncarriers and the ER22/23EK carriers. After the administration of 0.25 mg DEX insulin concentrations were not significantly different between the ER22/23EK carriers and the noncarriers (P = 0.11). Baseline (fasting) glucose levels tended to be lower in the ER22/23EK carriers than in the noncarriers (5.3 ± 0.20 and 5.6 ± 0.06 mmol/l, respectively; P = 0.07). To assess IR and insulin secretion, we used the HOMA-IR and HOMA for β-cell function (HOMA-B) index, respectively. As shown in Fig. 3*B*, at first examination, ER22/23EK carriers had significantly lower HOMA-IR scores (2.3 ± 0.33) than noncarriers (3.0 ± 0.11, $P < 0.05$). At the second measurement, the same pattern of HOMA-IR was observed (2.4 ± 0.39 vs. 3.8 ± 0.14 in ER22/23EK carriers and noncarriers, respectively; $P < 0.01$). HOMA-B scores tended to be lower in ER22/23EK carriers compared with noncarriers at both observations (84.8 ± 11.6 and 108.2 ± 3.5 for carriers and noncarriers, $P < 0.06$; and, at second examination, 119.0 ± 17.4 vs. 148.6 ± 4.7 carriers and noncarriers, $P <$ 0.07).

Risk factors for coronary heart disease and diabetes. In Table 3, serum concentrations of IGF-BP1, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides are shown. There were no differences between noncarriers and ER22/23EK carriers in IGF-BP1 levels or in HDL cholesterol and triglyceride concentrations. However, total cholesterol levels were significantly lower in ER22/23EK carriers than in noncarriers (6.86 ± 0.09 vs. 6.12 ± 0.25 mmol/l for noncarriers versus ER22/23EK carriers,

TABLE 2
Cortisol and DEX concentrations before and after 1 and 0.25 mg DEX, respectively, in noncarriers (n = 181 at first examination and n = 136 at second examination) and in ER22/23EK carriers (n = 17 at first examination and n = 13 at second examination)

	Noncarriers			ER22/23EK carriers			
	Mean	SE	Range	Mean	SE	Range	P
1 mg DST							
Fasting cortisol (nmol/l)	514.8	10.7	41–981	521.8	24.9	308–717	0.56
Post-DEX cortisol (nmol/l)	26.4	1.4	2–187	54.8	18.3	11–265	<0.0001
Δ cortisol (nmol/l)	484.5	10.3	39–856	467.0	31.7	138–674	<0.0001
DEX (nmol/l)	7.40	0.27	0.8–18.4	6.90	0.85	1.2–12.9	0.26
0.25 mg DST							
Fasting cortisol (nmol/l)	545.3	12.4	47–914	527.2	30.3	325–710	0.78
Post-DEX cortisol (nmol/l)	259.5	12.4	14–630	267.5	31.3	83–418	0.57
Δ cortisol (nmol/l)	285.8	13.5	−64–765	259.7	40.7	31–507	0.23
DEX (nmol/l)	2.85	0.13	0.1–8.7	2.88	0.52	0.6–7.1	0.69

Test for the difference between noncarriers and ER22/23EK carriers. All parameters were log transformed and adjusted for age and sex. Three subjects were taking estrogen-containing medication and were excluded from the analysis (one ER22/23EK carrier and two noncarriers). One noncarrier had not taken the 1-mg DEX tablet and was also excluded for that analysis.

TABLE 3
Risk factors for coronary heart disease and diabetes at first examination in noncarriers (n = 184) and ER22/23EK carriers (n = 18)

	Noncarriers			ER22/23EK carriers			
	Mean	SE	Range	Mean	SE	Range	P
IGF-BP1 (μg/l)	19.3	1.59	1–154	18.8	2.91	2.0–43.1	0.57
Total cholesterol (mmol/l)	6.86	0.09	2.80–10.32	6.12	0.25	4.31–7.41	0.02
LDL cholesterol (mmol/l)	5.11	0.08	0.91–8.31	4.31	0.25	2.65–6.09	0.01
HDL cholesterol (mmol/l)	1.36	0.03	0.72–2.51	1.43	0.14	0.84–2.83	0.63
Triglycerides (mmol/l)	1.91	0.07	0.47–7.42	1.93	0.33	0.76–7.00	0.67

Test for the difference between noncarriers and ER22/23EK carriers. All parameters were log transformed and adjusted for age.

$P = 0.02$), as well as LDL cholesterol levels (5.11 ± 0.08 vs. 4.31 ± 0.25 mmol/l in noncarriers versus ER22/23EK carriers, $P < 0.01$) At the second examination after 2.5 years, serum cholesterol concentrations were again lower (total cholesterol: 6.61 vs. 5.64 mmol/l in noncarriers versus ER22/23EK carriers, $P = 0.01$; LDL cholesterol: 4.87 vs. 3.87 mmol/l in noncarriers versus ER22/23EK carriers, $P < 0.01$ [not shown in table]).

Sex hormones. Table 4 shows the fasting concentrations of sex hormones for men and women separately. No differences between the noncarriers and the ER22/23EK carriers in the concentrations of estradiol, SHBG, androstenedione, DHEAS, or testosterone were detected. Again, the three subjects who were taking estrogen-containing medication were excluded from the analysis (one female ER22/23EK carrier and two female noncarriers).

Correlations. The degree of cortisol suppression after 1 and 0.25 mg DEX positively correlated with fasting insulin ($r = 0.19$, $P < 0.02$; and $r = 0.22$, $P < 0.03$, respectively) and HOMA-IR ($r = 0.21$, $P < 0.01$; and $r = 0.23$, $P < 0.02$, respectively), after adjustment for age and sex. These correlations also persisted after additional correction for BMI (Fig. 4). No correlations were found between a decrease in cortisol and insulin response, total HDL and LDL cholesterol, triglycerides, or HOMA-B scores. Baseline insulin concentrations correlated positively with triglycerides ($r = 0.31$, $P < 0.0001$), whereas an inverse relation was found with HDL cholesterol ($r = -0.24$, $P < 0.005$).

DISCUSSION

In this population study in the elderly involving 202 individuals, we found 18 subjects who were heterozygous for the ER22/23EK polymorphism (8.9%). Genotype distribution was in Hardy-Weinberg equilibrium, as far as we can say without finding any homozygous ER22/23EK carriers, which suggests that this sample was random. The ER22/23EK carriers had higher serum concentrations of cortisol and a smaller decrease in cortisol concentrations after the administration of 1 mg DEX than noncarriers. We would have expected to find a slight resistance more easily in a 0.25-mg DST than in a 1-mg test. We indeed found the same pattern of smaller decrease in cortisol and higher post-DEX cortisol levels in ER22/23EK carriers compared with noncarriers in the 0.25-mg DST. However, it was not significant, possibly because of the lower number of subjects who participated in the second test. Furthermore, ER22/23EK carriers tended to have lower insulin levels before and after a 1-mg DST. These data were partially confirmed 2.5 years later with a 0.25-mg DST. Fasting insulin concentrations were again lower in ER22/23EK carriers than in noncarriers, and fasting glucose levels tended to be lower in ER22/23EK carriers as well. In line with these data, HOMA-IR values were lower in ER22/23EK carriers, which indicates that they are more sensitive to insulin. These observations suggest that this polymorphism in the GR gene is associated with a slight resistance of the feedback regulation of the HPA axis. Furthermore, cortisol suppression after DEX correlated, in all individuals studied together, with fasting insulin and HOMA-IR, which was still significant after adjustment for BMI.

This relative resistance also results in a lower effect of cortisol on glucose metabolism, resulting in slightly lower glucose concentrations, as well as lower insulin levels. This favorable metabolic profile is supported by the observation that total and LDL cholesterol concentrations were significantly lower in the ER22/23EK group than in the group of noncarriers. This was confirmed at the second examination. These lower cholesterol concentrations can possibly be partially explained by a reduced

TABLE 4
Hormones at first examination in male (n = 89) and female (n = 93) noncarriers and in male (n = 9) and female (n = 8) ER22/23EK carriers

	Men					Women				
	Noncarriers		ER22/23EK carriers			Noncarriers		ER22/23EK carriers		
	Mean	SE	Mean	SE	P	Mean	SE	Mean	SE	P
Estradiol (pmol/l)	108.0	5.26	88.8	22.8	0.53	82.8	5.39	85.8	17.1	0.79
SHBG (nmol/l)	50.5	2.23	44.0	6.74	0.83	55.4	2.74	62.5	7.99	0.56
Androtenedione (nmol/l)	6.47	0.28	6.26	0.69	0.82	4.45	0.23	4.59	0.88	0.95
DHEAS (mmol/l)	3.97	0.24	3.80	0.46	0.76	2.38	0.49	2.54	0.16	0.92
Testosterone (nmol/l)	20.2	0.56	21.9	1.70	0.36	1.37	0.06	2.03	0.71	0.20

Shown are test results for the difference between noncarriers and ER22/23EK carriers. All parameters were log transformed. Three subjects were taking estrogen-containing medication and were excluded from the analysis (one female ER22/23EK carrier and two female noncarriers).

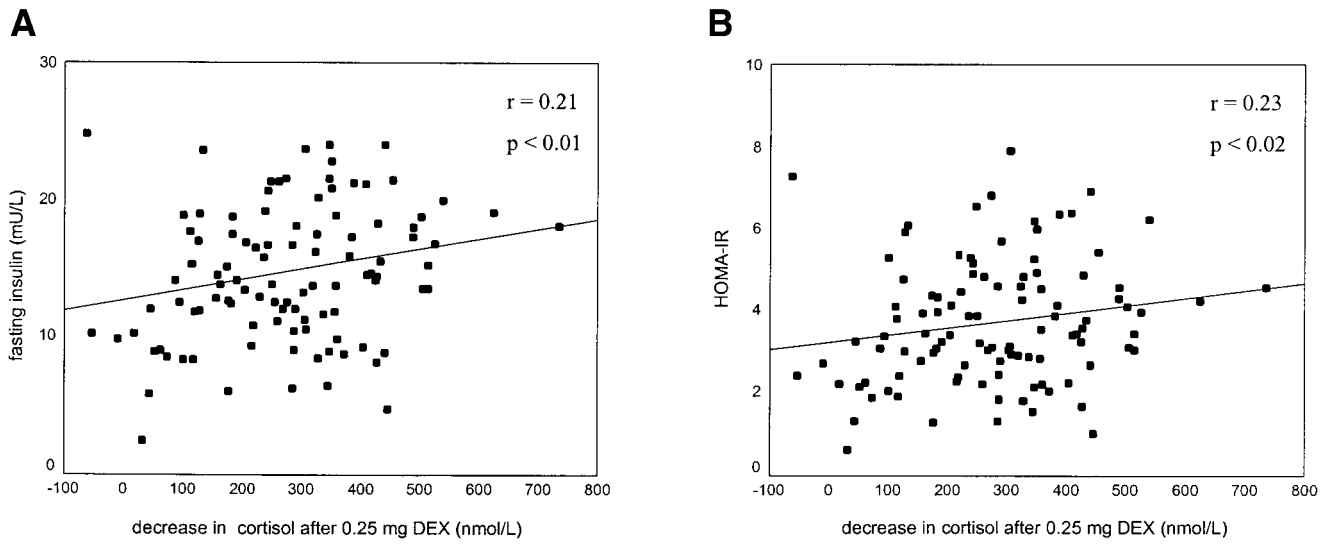

FIG. 4. Correlation between the decrease in serum cortisol concentrations after 0.25 mg DEX and fasting insulin (*A*) and HOMA-IR (*B*). A total of 179 individuals participated in the study. Data were adjusted for age, sex, and BMI. Subjects who had taken estrogen-containing medication or with fasting insulin >25 mU/l or fasting glucose >7.8 mmol/l were excluded from the analysis.

cortisol effect. GCs have been demonstrated to influence cholesterol levels by several mechanisms, such as regulation of the uptake of LDL by the liver (15) and influencing human adipose tissue lipoprotein lipase gene expression (16). These outcomes of a relative GC resistance, together with the lower insulin and total and LDL cholesterol and slightly lower serum glucose concentrations, indicate that ER22/23EK carriers have a healthier metabolic profile than noncarriers. In this respect, our observation of a significantly higher percentage of ER22/23EK carriers in the older age-group supports the finding of a beneficial metabolic effect of this GR polymorphism.

We found no other differences between the genotypes in anthropometric parameters, blood pressure, and serum levels of IGF-BP1, HDL cholesterol, triglycerides, or sex hormones. In particular, this last observation is probably in line with the fact that the ER22/23EK polymorphism in the GR gene is associated with a very mild degree of resistance, because other features previously described in patients with symptomatic GC resistance (2,3), such as hypertension and hypokalemia (related to compensatory adrenocorticotropic hormone–mediated mineralocorticoid overproduction) or acne and a male pattern of baldness (related to overproduction of adrenal androgens), were not observed. We have previously reported (17) five patients from our endocrine clinic who were diagnosed with cortisol resistance. At that time, to get a first impression of the effect of the ER22/23EK polymorphism, we had only genotyped 129 of the group of 216 subjects who underwent a DST. After completing the genotyping for the ER22/23EK polymorphism of the whole group, we report now subtle cortisol resistance, which is associated with this polymorphism. In our previous publication, we reported that 3 of the 129 genotyped individuals had post-DEX cortisol values >140 nmol/l. This cutoff value is clinically used to screen for Cushing's syndrome, but it seems less useful for studying cortisol sensitivity in the normal population. In the present study, we show that the 18 ER22/23EK carriers had a significantly smaller mean cortisol response to DEX, indicating a slight resistance to the negative feedback of cortisol. We also reported that two of five patients, who had a symptomatic cortisol-resistance syndrome, carried the ER22/23EK polymorphism (17). We cannot say whether this polymorphism was involved in the etiology of the syndrome, but it is possible that it was at least a factor, together with other causative factors.

Previously, we reported a polymorphism located in codon 363 in exon 2 of the GR, which was associated with an increased sensitivity to cortisol (10). The number of N363S carriers was not significantly different between the groups of ER22/23EK carriers and noncarriers (1 [5.5%] vs. 12 [6.5%] N363S carriers, $P = 0.32$). Moreover, exclusion of the N363S carriers did not alter the results.

In transient transfection assays in COS-1 cells, de Lange et al. (11) did not find differences in the way the 23K variant receptor regulated transcription from a number of different promoters. Although the polymorphism is not located in the core of the τ_1 transactivation domain, which is variably defined as amino acid 77–262 (18) or 98–305 (19), it is possible that the effects described here are caused by altered interactions with other proteins that do not play a role in the COS-1 system.

In summary, in this study we observed that subjects who were heterozygous for the 22/23EK allele had significantly higher post-DEX cortisol concentrations, a smaller decrease in cortisol concentrations, lower insulin, slightly lower fasting glucose levels, and slightly lower post-DEX insulin levels than subjects without this GR variant. Furthermore, ER22/23EK carriers had lower total and LDL cholesterol levels and were overrepresented in the older age-group. These data suggest that ER22/23EK carriers are relatively more "cortisol resistant" than noncarriers, which results in a better metabolic health profile. The exact mechanism through which the ER22/23EK variant of the GC receptor establishes these favorable effects remains unclear and is one of the subjects of our ongoing research.

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