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The ER22/23EK Polymorphism in the Glucocorticoid Receptor Gene Is Associated with a Beneficial Body Composition and Muscle Strength in Young Adults

ELISABETH F. C. VAN ROSSUM, PAUL G. VOORHOEVE, SASKIA J. TE VELDE, JAN W. KOPER, HENRIETTE A. DELEMARRE-VAN DE WAAL, HAN C. G. KEMPER, AND STEVEN W. J. LAMBERTS

Department of Internal Medicine (E.F.C.v.R., J.W.K., S.W.J.L.), Erasmus Medical Center, 3000 CA Rotterdam, The Netherlands; and Department of Pediatrics (P.G.V., H.A.D.-v.d.W.) and Institute for Research in Extramural Medicine (EMGO) (S.J.t.V., H.C.G.K.), Free University Medical Center, 1081 BT Amsterdam, The Netherlands

Glucocorticoids play an important role in determining body composition. A polymorphism of the glucocorticoid receptor gene (in codons 22 and 23) has previously been found to be associated with relative glucocorticoid resistance, low cholesterol levels, and increased insulin sensitivity. In this study, we investigated whether this ER22/23EK polymorphism is associated with differences in body composition and muscle strength. We studied a cohort of 350 subjects who were followed from age 13 until 36 yr. We compared noncarriers and carriers of the ER22/23EK variant in anthropometric parameters, body composition, and muscle strength, as measured by arm pull tests and high jump from standing. We identified 27 (8.0%) heterozygous ER22/23EK carriers. In males at 36 yr of age, we found that ER22/23EK carriers were taller, had more lean body mass, greater thigh circumference, and more muscle strength in arms and legs. We observed no differences in body mass index or fat mass. In females, waist and hip circumferences tended to be smaller in ER22/23EK carriers at the age of 36 yr, but no differences in body mass index were found. Thus, the ER22/23EK polymorphism is associated with a sex-specific, beneficial body composition at young-adult age, as well as greater muscle strength in males. (*J Clin Endocrinol Metab* 89: 4004–4009, 2004)

GLUCOCORTICOIDS ARE IMPORTANT regulators in numerous tissues throughout the human body, and they also influence body composition. Their effects are mainly mediated by the glucocorticoid receptor (GR), a ligand-activated transcription factor (1). Thus, changes in the gene coding for this receptor can play an important role in determining glucocorticoid sensitivity (2). Within the normal population, several polymorphisms in the *GR* gene have been described (3). One of these polymorphisms, N363S, was shown to be associated with an increased sensitivity to glucocorticoids and a higher body mass index (BMI) (4), as well as central obesity in males (5). Lin *et al.* (6) confirmed this finding of higher BMI in N363S carriers and showed an allele dosage effect of this polymorphism. In contrast, several other studies showed no effect on BMI (7, 8). A *Bcl*I polymorphism has previously been shown to be associated with a relative hypersensitivity to glucocorticoids *in vivo* (9), an increased cortisol response to a standardized lunch, and abdominal obesity in middle-aged subjects (10).

Previously, we identified another polymorphism that consists of two linked point mutations in codons 22 and 23 of the *GR* gene (GAG AGG→ GAA AAG). The first mutation in codon 22 is silent, with both GAG and GAA 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) (3). This polymorphism was associated with a relative resistance to glucocorticoids (11). We also showed in a population-based study in the elderly that carriers of this ER22/23EK polymorphism had a better insulin sensitivity and lower total and low-density lipoprotein cholesterol levels (11). In addition, we found the frequency of the 22/23EK allele to be higher in the elder half of the studied population, which suggests a survival advantage. To investigate whether the ER22/23EK variant is indeed associated with survival, we studied a separate population of 402 elderly Dutch men (12). After a follow-up of 4 yr, we found that 19.2% of the noncarriers had died, whereas none of the ER22/23EK carriers (n = 21) had died, which was a statistically significant difference. In this same population, we also found ER22/23EK carriers to have lower C-reactive protein levels, which in turn were also associated with a better survival. These lower C-reactive protein levels in ER22/23EK carriers possibly reflect a beneficial cardiovascular status (12).

A well-known effect of glucocorticoids is to negatively influence body composition, including redistribution of body fat with deposition of adipose tissue on the abdomen and trunk, and muscle atrophy (13). It is known that body composition plays an important role in lipid metabolism and insulin sensitivity and, as a consequence, influences the risk on cardiovascular disease (14). At present, it is not known what the effects of this ER22/23EK polymorphism are at a young age or whether there are any effects on body composition.

Therefore, in the present study, we investigated a cohort

Abbreviations: BMI, Body mass index; GR, glucocorticoid receptor; MET, metabolic equivalent.

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of 350 subjects who were followed from 13 until 36 yr of age and studied whether there is an association between the ER22/23EK polymorphism of the *GR* gene and body composition during puberty and at young-adult age.

Subjects and Methods

Subjects

Healthy participants (350) were drawn from the Amsterdam Growth and Health Longitudinal Study, a population-based observational study with repeated measurements at the age of 13, 14, 15, 16, 27, 29, 32, and 36 yr (15). Subjects of non-Caucasian race were excluded from the analyses (five males and five females, all noncarriers of the ER22/23EK polymorphism). For a total of 337 (158 males), we had complete data on GR genotype and adult anthropometry. Data were not complete at all measurements in puberty: a total of 332 subjects (26 ER22/23EK carriers) participated at 13 yr, 290 (22 ER22/23EK carriers) at 14 yr, 286 (21 ER22/23EK carriers) at 15 yr, and 287 (19 ER22/23EK carriers) at 16 yr of age. All subjects gave their written informed consent to participate in the study, which received the approval of the Medical Ethical Committee of Vrije Universiteit (Amsterdam, The Netherlands).

Anthropometric measurements

Body weight (kilograms) was measured to the nearest 0.1 kg using a spring balance scale (Van Vucht, Amsterdam, The Netherlands), with subjects dressed only in underwear. Standing height was measured with a stadiometer to the nearest 0.001 m. BMI was calculated as body weight divided by body height squared. To assess fat distribution (abdominal *vs.* gluteo-femoral), we measured waist (at the umbilicus) and hip circumference with a flexible steel tape to the nearest 1 mm, and the waist to hip ratio was calculated. Fat mass was estimated from four skinfold thickness measurements (biceps, triceps, subscapular, and supra iliacal) by the equation of Durnin *et al.* (16–18). Lean body mass was measured by dual-energy x-ray absorptiometry with the Hologic QDR-2000 (S/N 2513; Hologic, Inc., Waltham, MA). Calf and thigh circumferences were measured with a steel tape to the nearest 0.1 cm.

Muscle strength

Muscle strength was assessed by two physical fitness tests from the Motor Performance (MOPER) fitness test battery (19, 20). The first was the static arm pull test: the subjects were given two attempts to pull maximally with their arm of preference, the strength of which was measured (in kilograms) with a dynamometer (Bettendorf, Brussels, Belgium), fixed to the wall at a horizontal level. The higher score of the two was recorded. The second test was the standing high jump. The subjects had two attempts to jump as high as possible (higher value recorded) from a platform, having been allowed only to bend their knees before jumping. The height they jumped (in centimeters) was measured by a tape, which was fixed to a belt around a subject's waist to the platform on the ground.

Physical activity

A structured interview based on a physical activity questionnaire was used to investigate the amount of physical activity. The questionnaire comprises questions about duration, frequency, and metabolic equivalent (MET) intensity of all physical activities during the last 3 months preceding the interview. From this information, a total weighted activity score (METs per week) was calculated (21, 22).

Genetic analysis

Restriction fragment length polymorphism analysis was carried out to determine GR genotypes. DNA was extracted from peripheral blood leukocytes by standard techniques. PCR amplification of the GR gene was carried out using primer sequences and amplification conditions as described previously (3). The PCR products were digested with 1 U *Mnl*I (PerkinElmer Life and Analytical Sciences, Boston, MA) 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 (Roche Diagnostics, Mannheim, Germany). We reanalyzed all heterozygous and 10 wild-type samples and found identical genotypes.

Statistical analysis

Data were analyzed by SPSS for Windows, release 10.1 (SPSS, Chicago, IL). Differences in means between the ER22/23EK carriers and the noncarriers were adjusted for height if appropriate and tested by analysis of covariance using the general linear model procedure. High jump scores were corrected for body weight. Results are reported as mean ± SE. P values are two-sided throughout, and $P \leq 0.05$ was considered to indicate a significant difference.

Results

Anthropometric parameters at young-adult age

In the group of 337 participants, we identified 27 (8%) carriers of the ER22/23EK polymorphism (16 males and 11 females). Table 1 shows anthropometric parameters determined in noncarriers and carriers of the ER22/23EK polymorphism at the last measurement (at the age of 36 yr).

TABLE 1. Mean and SE of anthropometric parameters, muscle strength, and MET activity score in noncarriers of both sexes and in male (n = 16) and female (n = 11) ER22/23EK carriers at the age of 36 yr

	Men (n = 158)					Women (n = 179)				
	Noncarriers		ER22/23EK carriers			Noncarriers		ER22/23EK carriers		
	Mean	SE	Mean	SE	P	Mean	SE	Mean	SE	P
Height (m)	1.83	0.01	1.87	0.02	0.05	1.71	0.01	1.70	0.02	0.48
Weight (kg)	83.5	0.9	89.8	3.1	0.14	68.5	0.8	63.2	1.8	0.13
BMI (kg/m^2)	24.8	0.2	25.7	0.8	0.20	23.5	0.3	22.0	0.8	0.18
Total fat (%)	21.5	0.5	21.1	2.0	0.87	32.3	0.6	30.6	2.1	0.36
Total fat mass (kg)	18.1	0.6	19.5	2.4	0.66	22.1	0.6	19.4	1.7	0.26
Total lean mass (kg)	61.4	0.5	66.2	1.5	0.02	42.7	0.4	41.0	1.2	0.38
Thigh circumference (cm)	57.4	0.4	60.4	1.1	0.03	57.2	0.4	55.7	1.0	0.38
Calf circumference (cm)	37.8	0.2	38.7	0.9	0.31	36.1	0.2	35.6	0.6	0.56
Upper arm circumference (cm)	30.5	0.2	31.2	7.3	0.45	27.6	0.2	26.3	0.7	0.15
Waist circumference (cm)	85.0	0.7	87.5	2.1	0.43	73.5	0.7	68.6	1.3	0.07
Hip circumference (cm)	89.0	0.6	91.9	1.8	0.31	89.3	0.7	84.7	2.2	0.09
Arm pull (kg)	70.9	1.1	77.8	3.9	0.06	38.8	0.6	36.2	2.2	0.32
High jump (cm)	51.9	0.6	55.7	1.8	0.04	38.7	0.4	40.1	2.5	0.64
MET score (METS/wk)	4243	267	4678	796	0.60	5316	297	6119	1733	0.49

Test for the difference between noncarriers and ER22/23EK carriers. All parameters, except the arm pull strength, high jump from standing, and MET score, were corrected for height. High jump scores were adjusted for body weight.

In males, we found a greater body height in ER22/23EK carriers ($P = 0.05$), as well as a higher body weight ($P = 0.03$). However, the latter was not significant after adjustment for height ($P = 0.14$). BMI was not different between the genotypes. Total lean mass was significantly higher in ER22/23EK carriers compared with noncarriers (66.2 ± 1.5 and 61.4 ± 0.5 kg, respectively; $P = 0.006$, after additional correction for height $P = 0.02$). The circumference of the thigh was also greater in ER22/23EK carriers (ER22/23EK, 60.4 ± 1.1; and noncarriers, 57.4 ± 0.4 cm; $P = 0.03$), whereas no differences were found in total fat mass or percentage fat.

Table 1 also shows anthropometric parameters in female noncarriers and ER22/23EK carriers at the age of 36 yr. In females, body weight tended to be lower in ER22/23EK carriers (63.2 ± 1.8; and noncarriers, 68.5 ± 0.8 kg), although this was not statistically significant after adjustment for height ($P = 0.13$). BMI was also not significantly different between the two genotypes (ER22/23EK, 22.0 ± 0.8; and noncarriers, 23.5 ± 0.3; $P = 0.18$). Waist and hip circumferences tended both to be lower in female ER22/23EK carriers compared with noncarriers ($P = 0.07$ and 0.09, respectively, Table 1). No differences were found in height, fat mass, and lean body mass, or circumferences of the thigh, calf, and upper arm.

At the age of 32 yr, we found similar results (not shown in Table 1). Male ER22/23EK carriers had a greater body height ($P = 0.035$, Fig. 1A), higher lean body mass ($P = 0.02$, Fig. 1B), and higher weight ($P = 0.006$; after adjustment for height, $P = 0.08$), whereas total fat mass was not different ($P = 0.12$).

In females, we found a tendency toward a smaller waist circumference in female ER22/23EK carriers (ER22/23EK, 67.5 ± 1.4; and noncarriers, 71.1 ± 0.5; $P = 0.08$). No differences in hip circumference (ER22/23EK, 87.2 ± 2.1; and noncarriers, 90.1 ± 0.7; $P = 0.27$) or in height, weight, BMI, body composition, and muscle strength were observed at the age of 32 yr.

Anthropometric parameters in puberty

During puberty, we also measured anthropometric variables, body composition, and muscle strength in the same subjects. Figure 1A shows the height of male noncarriers and carriers of the ER22/23EK polymorphism during puberty. Although the pattern of greater height in male ER22/23EK carriers is similar to that at adult age, these differences were not statistically significant. The same applied to the amount of lean mass in males: no significant differences during puberty between genotypes, although a similar pattern as at adult age (higher lean mass in male ER22/23EK carriers) could be observed (Fig. 1B). At the age of 15 yr, we found tendencies toward higher body weight ($P = 0.10$), BMI ($P = 0.06$), and lean mass (0.09) in ER22/23EK carriers. On average, male noncarriers grew an additional 5.9 cm after the age of 16 yr, whereas ER22/23EK carriers grew 7.8 cm until they reached their final height; however, this was not a significant difference. In males, no differences were found in other anthropometric parameters or body composition variables during this period. In females, no differences were observed in the measured parameters at these four measurements during puberty.

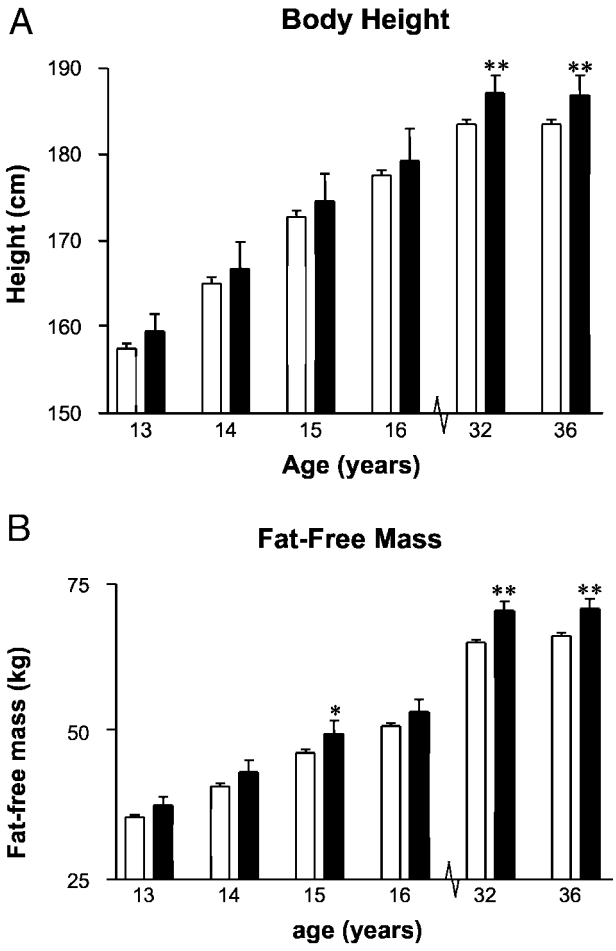

FIG. 1. Height (A) and fat-free mass (B) in male noncarriers (*white bars*) and carriers of the ER22/23EK polymorphism (*black bars*) during puberty (13, 14, 15, and 16 yr) and adult age (32 and 36 yr). **, $P \leq 0.05$; *, $P < 0.10$.

Muscle strength at adult age and during puberty

Male ER22/23EK carriers tended to perform better in the test of arm pull strength ($P = 0.06$; Fig. 2A), as well as in high jump from standing (adjusted for body weight, $P = 0.04$, Fig. 2B) at the age of 36 yr (see also Table 1). Arm pull strength was significantly greater in males at the age of 32 yr (Fig. 2A; ER22/23EK, 81.2 ± 3.3; and noncarriers, 73.0 ± 1.1; $P = 0.02$). During puberty, we found the same tendencies toward better arm strength in male ER22/23EK carriers (Fig. 2A). Performance on high jump from standing was not significantly different between the genotypes in males at the age of 32 yr or during puberty (Fig. 2B). In females, we did not observe any differences in muscle strength of the arm or leg at the age of 36 or 32 yr or during puberty.

Physical activity

At both measurements at young-adult age, MET scores were determined to evaluate physical activity in daily life of the participants. No differences in MET scores between the

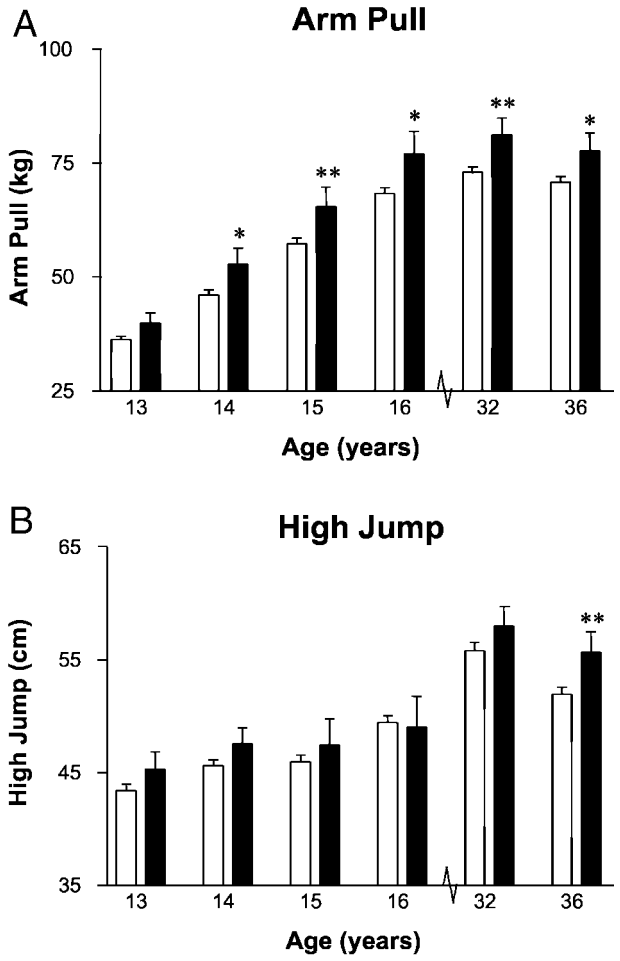

FIG. 2. Arm pull strength (A) and standing high jump (B) in male noncarriers (*white bars*) and carriers of the ER22/23EK polymorphism (*black bars*) during puberty (13, 14, 15, and 16 yr) and adult age (32 and 36 yr). High jump scores were corrected for body weight. **, $P \leq 0.05$; *, $P < 0.10$.

genotypes were observed in both sexes at ages 36 yr (males, $P = 0.60$; and females, $P = 0.49$; Table 1) and 32 yr (males, ER22/23EK, 2755 ± 279 and noncarriers, 3236 ± 255 METS/wk, $P = 0.52$; and females, ER22/23EK, 3687 ± 795 and noncarriers, 3547 ± 202 METS/wk, $P = 0.85$).

Discussion

In this population-based cohort study in young subjects, we identified 8% heterozygous carriers of the ER22/23EK polymorphism. In males at young-adult age, we found ER22/23EK carriers to be on average 4 cm taller than noncarriers and to have significantly more lean body mass, whereas there were no differences in fat mass. In addition, male ER22/23EK carriers had greater thigh circumferences, indicating more muscle mass. Functional muscle strength tests showed a better performance of ER22/23EK carriers in arm strength, with the greatest difference at the age of 32 yr, as well as a better performance in tests concerning strength of the legs. MET scores did not differ between genotypes, so differences in physical activity did not underlie the greater amount of muscle mass in male ER22/23EK carriers.

These differences in body composition in males were not yet clearly present during puberty. However, a tendency could be observed toward greater arm strength in male ER22/23EK carriers during this period, which suggests that the differences already might have existed in puberty. The lack of statistical significance of the other body compositional parameters could possibly be due to the lower numbers of subjects who participated at pubertal age. These incomplete data during puberty might also explain a minor part of the difference in mean height between the ages of 16 and 32 yr. However, most of this difference in height is explained by natural growth. In The Netherlands, boys grow an additional 5.5 cm after the age of 16 yr. Interestingly, male ER22/23EK carriers grew on average almost 2 cm more than noncarriers after the age of 16 yr. This increased growth suggests that puberty in ER22/23EK carriers might be extended compared with noncarriers. It is known that glucocorticoids inhibit growth during puberty. Because we found the ER22/23EK polymorphism to be associated with relative glucocorticoid resistance, we would expect less inhibition of growth and thus a greater height. This is in accordance with our finding of a greater height in male carriers of the ER22/23EK polymorphism. Taken together, it remains unclear at what developmental stage exactly these differences between the genotypes that we observed in young adults in height, lean mass, and thigh circumference arise. Although the mean heights in this population-based study appear rather tall, these heights are in accordance with the mean height at these ages in The Netherlands. This suggests that our findings are very well applicable to the Dutch population as a whole.

In young-adult females, we found in ER22/23EK carriers tendencies toward smaller waist and hip circumferences and lower body weight, suggesting a lower amount of sc fat. These differences could not be detected during puberty. No statistically significant differences were found in measures of body composition or muscle strength between the genotypes.

Long-term exposure to high levels of glucocorticoids are known to negatively influence muscle mass and growth (23, 24). Thus, the findings of greater height and more muscle mass in male ER22/23EK carriers could be explained by the observation that ER22/23EK carriers are relatively resistant to the effects of glucocorticoids, as we recently demonstrated (11). Another well-known chronic effect of glucocorticoids is redistribution of fat mass to the abdominal region. In line with a glucocorticoid-insensitive effect of the ER22/23EK polymorphism, we found at the ages of 32 and 36 yr a tendency toward smaller waist circumference in female ER22/23EK carriers.

The associations between the ER22/23EK polymorphism and body composition appear to be different between sexes. However, there could be subtle anabolic effects in female ER22/23EK carriers as well, in line with a relative cortisol resistance and as a result possibly higher androgen levels. When we consider the mean weight difference (>5 kg) between female noncarriers and carriers of the ER22/23EK polymorphism, the difference in lean body mass is quite small (<2 kg), which indicates that female ER22/23EK carriers also have relatively more lean body mass. However, the number of female carriers of the polymorphism is relatively

small, which might explain that we found no statistically significant differences. Besides more muscle mass, we would also expect less fat mass in subjects with slightly higher androgen levels. In females, we observed tendencies toward smaller waist and hip circumferences, which might reflect a lesser amount of sc fat mass.

On the other hand, the ER22/23EK polymorphism could have sex-specific effects on body composition. We speculate that differential effects of sex steroid hormones and/or growth hormone could play a role. It is known that, in rodents, the hypothalamic-pituitary-adrenal axis is differently regulated in males and in females, both in basal conditions and in response to psychological or physical stress conditions (25). In this context, androgens inhibit and estrogens enhance the hypothalamic-pituitary-adrenal responsiveness to stress (26, 27). In addition, in a relative glucocorticoid-resistant condition, as is the case in carriership of the ER22/23EK variant allele, ACTH production is expected to be slightly higher than in noncarriers due to the lower negative feedback inhibition at the pituitary level. As a consequence, ER22/23EK carriers might have slightly higher circulating androgen concentrations, which could also, besides a smaller direct (negative) effect of glucocorticoids, contribute to the observed beneficial body composition. The differential effects of sex steroid hormones might explain the gender dimorphism in the associations we observed between genotype and body composition. However, in the present study, we did not measure any serum hormone concentrations.

The exact mechanism of this polymorphism at the molecular level is unknown. The amino acid change in codon 23 (arginine to lysine) might affect the tertiary structure of the receptor. Because the ER22/23EK variant is located near the transactivation domain, this could influence the transactivational and/or transrepressional activity on target genes (28, 29). Recently, it has been shown that two different GR isoforms (A and B) exist, due to two different methionine (M) codons in the GR mRNA, which both can be used as imitation codon (M1 and M27). The GR-B protein has a stronger transactivating effect in transient transfection experiments but no difference in transrepression (30). The secondary structure of the GR mRNA might be affected by the ER22/23EK polymorphism, which could result in a different usage of the initiation codons. A change in GR-A to GR-B ratio could then explain the decreased sensitivity to glucocorticoids (29). Indeed, secondary structure prediction (M-fold) showed different structures for the wild-type and polymorphic mRNA (29). A third possibility is that the ER22/23EK polymorphism might lead to differences in binding of proteins, which could affect mRNA stability and thereby influence glucocorticoid sensitivity.

In summary, we found that the ER22/23EK polymorphism of the *GR* gene is associated with greater body height and more muscle mass and strength in young-adult males. In females, we found a tendency toward smaller waist circumference and to a lesser extent smaller hip circumference. Thus, we conclude that the ER22/23EK polymorphism is associated with a sex-specific, beneficial body composition at young-adult age, as well as more muscle strength in males.

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Address all correspondence and requests for reprints to: Elisabeth F. C. van Rossum, M.D., Laboratory of Internal Medicine, Room Ee 585 B, Erasmus Medical Center, Dr. Molewaterplein 50, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands. E-mail: e.vanrossum@erasmusmc.nl.

E.F.C.v.R., P.G.V., and S.J.t.V. contributed equally.

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