Association of the ER22/23EK Polymorphism in the Glucocorticoid Receptor Gene with Survival and C-Reactive Protein Levels in Elderly Men

Elisabeth F. C. van Rossum, MD, Richard A. Feelders, MD, PhD, Annewieke W. van den Beld, MD, PhD, André G. Uitterlinden, PhD, Joop A. M. J. L. Janssen, MD, PhD, Wietske Ester, MD, Albert O. Brinkmann, PhD, Diederick E. Grobbee, PhD, Frank H. de Jong, PhD, Huibert A. P. Pols, MD, PhD, Jan W. Koper, PhD, Steven W. J. Lamberts, MD, PhD

PURPOSE: We recently demonstrated that a polymorphism in codons 22 and 23 of the glucocorticoid receptor gene is associated with relative glucocorticoid resistance, greater insulin sensitivity, and lower total and low-density lipoprotein cholesterol levels. In the present study, we investigated whether the ER22/23EK polymorphism is associated with survival, cholesterol levels, and two predictors of mortality: serum C-reactive protein and interleukin 6 levels.

METHODS: We studied 402 men (mean [± SD] age, 77.8 ± 3.6 years). C-reactive protein was measured by a highly sensitive method using a latex-enhanced immunoephelometric assay. Interleukin 6 was determined by a commercially available immulite assay.

RESULTS: After a follow-up of 4 years, 73 (19%) of 381 noncarriers died, while none of the 21 ER22/23EK carriers had died ($P = 0.03$). C-reactive protein levels were about 50% lower in ER22/23EK carriers ($P = 0.01$). There were no differences in interleukin 6 levels.

CONCLUSION: Carriers of the ER22/23EK polymorphism have better survival than noncarriers, as well as lower C-reactive protein levels. **Am J Med. 2004;117:158–162.** ©2004 by Elsevier Inc.

Most of the effects of glucocorticoids are mediated by the glucocorticoid receptor (1). Recently, we demonstrated that a polymorphism in codons 22 and 23 of the glucocorticoid receptor gene (GAGAGG [GluArg, or ER] → GAAAAG [GluLys, or EK]) is associated with relative glucocorticoid resistance (2). This ER22/23EK variant was also associated with greater insulin sensitivity and lower total and low-density lipoprotein (LDL) cholesterol levels. Furthermore, we found that the number of ER22/23EK carriers was significantly higher in the older half of the sample, suggesting that the polymorphism had a beneficial effect on survival.

The ER22/23EK variant might also affect the inflammatory response, and elevated levels of two inflammatory markers—C-reactive protein and interleukin 6—are associated with mortality in the elderly (3). C-reactive protein is an independent risk factor for cardiovascular events (4–7). Interleukin 6 stimulates the synthesis of C-reactive protein, as well as other acute phase proteins in the liver, and is elevated in patients with unstable angina or heart failure (8–11). Therefore, we investigated whether C-reactive protein and interleukin 6 levels, as well as survival, were associated with the ER22/23EK polymorphism of the glucocorticoid receptor gene.

METHODS

Subjects

We recruited 402 men, aged 73 years or older, by a letter that was sent to the oldest men in Zoetermeer, The Netherlands. Subjects were eligible to participate if they were physically and mentally able to visit the study center independently. No additional health-related criteria were used. Medications taken for more than 6 months were recorded. Data on vital status of the participants and causes of death during 4-year follow-up were obtained by contacting the participants' general practitioners. Before the start of the study, which received the approval of the Medical Ethics Committee of the Erasmus Medical Center, all subjects had given their written informed consent to participate.

Measurements

Weight and height were measured, and the body mass index (kg/m^2) was calculated. Blood pressure was measured in sitting position at the right upper arm with a random-zero sphygmomanometer. Total fat mass, trunk fat mass, and lean body mass were measured using dual-energy X-ray absorptiometry (Lunar Corp., Madison,

From the Departments of Internal Medicine (EvR, RF, AvdB, AU, JJ, WE, FdJ, HP, JK, SL) and Reproduction and Development (AB), Erasmus Medical Center, Rotterdam, The Netherlands; and Julius Center (DG), University of Utrecht, The Netherlands.

This research project was supported by a grant from NWO and Research Institute of Diseases in the Elderly.

Requests for reprints should be addressed to Elisabeth F. C. van Rossum, MD, Department of Internal Medicine, Room Ee 585 B, Erasmus Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands, or e.vanrossum@erasmusmc.nl.

Manuscript submitted June 17, 2003, and accepted in revised form January 29, 2004.

0002-9343/04/$–see front matter
doi:10.1016/j.amjmed.2004.01.027

Wisconsin) (12). Quality assurance, including calibration, was performed every morning, using the standard provided by the manufacturer.

Levels of total and high-density lipoprotein (HDL) cholesterol and triglycerides were measured using standard laboratory methods; LDL cholesterol levels were calculated. C-reactive protein was measured with a highly sensitive method using a latex-enhanced immunoephelometric assay on a BN II analyser (Dade Behring, Liederbach, Germany). Interleukin 6 was determined by a commercially available immulite assay (Diagnostic Products Corporation, Los Angeles, California), using aliquots of undiluted sera tested against an absolute interleukin 6 standard preparation. Based on these standard curves, the concentrations of interleukin 6 were calculated by the software provided. Cortisol was measured using a radioimmunoassay (Diagnostic System Laboratories, Webster, Texas).

Lower extremity function or physical performance was assessed with measurements of standing balance, walking speed, and ability to rise from a chair (13). A summary performance scale, which ranged from 0 (worst) to 12 (best), was created by summing these scores. Satisfaction in performing activities of daily living was assessed by using a self-administered questionnaire (14). All items are evaluated on a 4-point scale; higher scores denote greater impairment.

At baseline, a 21-item medical history was obtained by a structured questionnaire, according to the following groups: musculoskeletal impairments (including arthritis and fractures); cardiovascular impairments (including symptoms or treatment of angina pectoris, heart failure, hypertension, arrythmia, myocardial infarction, cerebrovascular accident, and shortness of breath); prostate problems (hyperplasia and cancer); other malignancies; endocrine disorders (diabetes mellitus and thyroid disease); and other conditions (dizziness and disturbed vision that impair mobility). A physical examination was performed. None of the participants was being treated for systemic infectious, inflammatory, or malignant disorders at the time of enrollment.

Glucocorticoid receptor genotypes were determined by restriction fragment length polymorphism analysis (15). For confirmation, we reanalyzed all 21 heterozygous samples and 10 wild-type samples, and found identical genotypes.

Statistical Analysis

Data were analyzed using SPSS for Windows, release 10.1 (SPSS, Chicago, Illinois). Differences between the ER22/23EK carriers and the noncarriers were adjusted for age and, if necessary, for body mass index or smoking and tested by analysis of covariance using the general linear model procedure. Bonferroni post hoc tests were used to adjust for multiple comparisons. If dependent variables were not normally distributed, logarithmic transformations were applied to normalize them or nonparametric tests (Mann-Whitney U test) were used. Continuous variables are reported as mean ± SD or median with the interquartile range. Survival was analyzed using the Kaplan-Meier procedure and log-rank test. To study the association between C-reactive protein levels and mortality, C-reactive protein levels were divided in two groups (high vs. low, based on the median value). Cox proportional hazards models were used to analyze this relation, adjusting for genotype, diabetes, and health status. Correlations between C-reactive protein levels and parameters of body composition and cortisol levels were calculated using Spearman's correlation. $P < 0.05$ was considered statistically significant.

RESULTS

Of the 402 men, 21 (5%) were heterozygous for the ER22/23EK polymorphism. No homozygotes were found. There were no significant differences in age, smoking status, measures of body composition, or lipids between the carriers and noncarriers (Table).

Mortality

Of the 381 noncarriers, 73 (19%) died during the 4 years of follow-up, while none of the 21 ER22/23EK carriers had died (Figure, $P = 0.03$). Causes of mortality included cardiovascular disease (40%, n = 29), cancer (11%, n = 8), pneumonia (7%, n = 5), cerebrovascular accident (5%, n = 4), miscellaneous (cachexia, infections, pulmonary emphysema: 7%, n = 5), and unknown (30%, n = 22). Men with lower C-reactive protein levels (less than the median) had significantly better survival (13% [27/201] died) than those with higher C-reactive protein levels (24% [47/201] died; hazard ratio = 1.8; 95% confidence interval: 1.1 to 2.9). Additional analyses that adjusted for genotype, diabetes, or general health status (as physical performance, activities of daily life scores, and morbidity data) did not change these results.

Anthropometric, Metabolic, and Inflammatory Parameters in ER22/23EK Carriers and Noncarriers

ER22/23EK carriers had significantly lower C-reactive protein levels than noncarriers (Table). Lean body mass tended to be higher in ER22/23EK carriers compared with noncarriers; however, this did not reach statistical significance after correction for height. C-reactive protein levels correlated significantly with body mass index ($r = 0.16$, $P = 0.002$), total fat mass ($r = 0.15$, $P = 0.003$), and trunk fat mass ($r = 0.16$, $P = 0.001$), but not with lean body mass ($r = 0.02$, $P = 0.63$). Early morning cortisol levels did not correlate with C-reactive protein levels ($r = 0.04$, $P = 0.48$) or genotype ($P = 0.81$).

Table 1. Baseline Characteristics of Noncarriers and Carriers of the ER22/23EK Polymorphism among 402 Elderly Men*

Characteristic	Noncarriers (n = 381)	ER22/23EK Carriers (n = 21)	*P* Value
	Number (%), Mean ± SD, or Median (Interquartile Range)		
Age (years)	77.7 ± 3.6	78.3 ± 3.6	0.46
Smokers	65 (17)	5 (24)	0.43
Body mass index (kg/m^2)	25.6 ± 4.3	25.9 ± 3.1	0.75
Lean mass (kg)	51.7 ± 5.6	52.8 ± 5.2	0.18†
Fat mass (kg)	21.1 ± 5.7	21.2 ± 6.6	0.89†
Trunk fat mass (kg)	10.6 ± 2.6	10.6 ± 2.9	0.98
Systolic blood pressure (mm Hg)	56 ± 24	158 ± 29	0.67‡
Diastolic blood pressure (mm Hg)	84 ± 11	86 ± 13	0.49‡
Total cholesterol (mmol/L)§	5.8 ± 1.1	5.4 ± 1.0	0.14‡
LDL cholesterol (mmol/L)§	3.8 ± 1.0	3.5 ± 0.9	0.22‡
HDL cholesterol (mmol/L)§	1.3 ± 0.4	1.3 ± 0.3	0.36‡
Triglycerides (mmol/L)§	1.4 ± 0.8	1.3 ± 0.7	0.75‡
C-reactive protein (mg/L)§	4.0 ± 9.1	2.0 ± 2.6	0.01
Interleukin 6 (pg/mL)	19.1 ± 11.1	19.9 ± 9.2	0.47
Diabetes	30 (8)	3 (14)	0.31
Activities of daily life (points)	9 (8–12)	9 (8–11)	0.88
Physical performance (points)	9 (7–10)	9 (7–11)	0.54
Number of chronic diseases	3 (1–5)	3 (1–5)	0.78

* All parameters were adjusted for age, and C-reactive protein and interleukin 6 values were logarithmically transformed for statistical analysis.
† Lean mass and fat mass were also adjusted for height.
‡ Blood pressures and lipids were adjusted for body mass index.
§ To convert total, HDL, and LDL cholesterol levels from mmol/L to mg/dL, multiply by 38.67; for triglyceride levels, multiply by 89.15. To convert C-reactive protein levels from mg/L to mg/dL, divide by 10. HDL = high-density lipoprotein; LDL = low-density lipoprotein.

We found no differences in body mass index, blood pressure, or levels of HDL cholesterol, triglycerides, or interleukin 6 between ER22/23EK carriers and noncarriers (Table).

DISCUSSION

In this population-based study involving elderly men, we found that carriers of the ER22/23EK polymorphism of the glucorticoid receptor gene had better 4-year survival. This is in line with our previous findings (2), in which we observed that the proportion of ER22/23EK carriers was significantly greater in the older half of the study sample. The ER22/23EK carriers also had significantly lower C-reactive protein levels, but no association was found with anthropometric parameters or interleukin 6 levels. Since C-reactive protein level predicts total and cardiovascular mortality in the elderly (16), it might underlie the effect of the ER22/23EK polymorphism on survival. However, it is unclear whether C-reactive protein is a cause of atherosclerosis or whether it reflects the degree of vascular damage (17). We previously demonstrated that carriers of the ER22/23EK polymorphism are relatively resistant to the effects of glucocorticoids, and have lower fasting insulin and cholesterol levels (2). Perhaps the lower C-reactive protein levels we observed in ER22/23EK carriers are related to, and possibly even due to, having less atherosclerosis.

Greater body mass index, and fat mass in particular, are related to higher levels of interleukin 6 and C-reactive protein (18–22). We found that C-reactive protein level correlated positively with body mass index and fat mass in these elderly men, but there was no association between interleukin 6 and these parameters. We did not observe differences in body mass index or fat mass by genotype. ER22/23EK carriers had a slightly (but not significantly) higher lean body mass. We hypothesize that a greater lean body mass, which might also result in greater insulin sensitivity, may also be a factor in the better cardiovascular health status of ER22/23EK carriers, thereby contributing to lower C-reactive protein levels. In another study, we found associations of the ER22/23EK polymorphism with body composition in young adults, specifically in male carriers aged 36 years with an average of 5 kg more lean mass and in female carriers with tendencies towards less fat mass. Muscle mass is an impor-

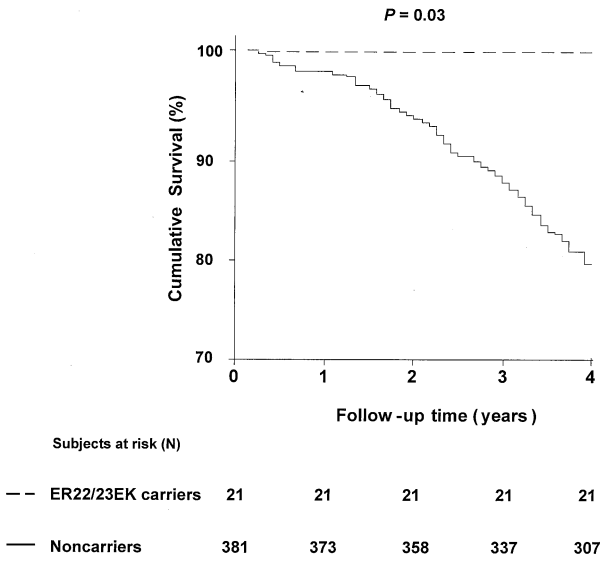

Figure. Kaplan-Meier survival curves by glucocorticoid receptor genotype. ER22/23EK carriers had significantly better survival than noncarriers. The solid line denotes noncarriers; the dotted line denotes ER22/23EK carriers.

tant determinant of insulin sensitivity and can thus contribute to a better metabolic profile. We believe that body composition could play an important role in the relation of the ER22/23EK polymorphism with both survival and C-reactive protein levels.

Interleukin 6 is a potent stimulator of C-reactive protein in the liver and acts synergistically with glucocorticoids to induce the synthesis of other acute phase proteins by the liver. In addition, glucocorticoids inhibit the production of interleukin 6 when administered in pharmacological amounts or when present at high levels (23,24). However, in physiological circumstances, the stimulatory effect of glucocorticoids on interleukin 6 is minor or even absent (23). The variation in basal cortisol levels in carriers and noncarriers of the ER22/23EK polymorphism is likely to be within the physiological range because cortisol levels did not differ significantly by genotype. However, in general, the biological response of a target cell to a hormone is determined by several factors, including the concentration of the hormone, the concentration of receptors, and the affinity of the hormone-receptor interaction. For example, glucocorticoids also upregulate the interleukin 6 receptor (25), by which they can influence the biological effects of interleukin 6. The ER22/23EK polymorphism, which is associated with relative resistance to the effects of glucocorticoids, might result in a lesser degree of upregulation of the interleukin 6 receptor. Thus, although there were no differences in circulating interleukin 6 concentrations, the ER22/23EK polymorphism might result in a decreased stimulation of C-reactive protein production. In addition, C-reactive protein can be synthesized by adipocytes without mediation of interleukin 6 (26).

Thus, the association between ER22/23EK polymorphism and mortality might be due to factors other than C-reactive protein. We reported an association between the ER22/23EK polymorphism and greater insulin sensitivity, as well as lower total and LDL cholesterol levels in a population-based sample that had a mean age of 67 years. Although we observed a similar pattern in the present study, we did not find statistically significant differences in cholesterol levels, perhaps because the mean age in the current study was more than 10 years older; selection of surviving participants with relatively low cholesterol levels may have occurred.

In this study, and in our previous study (2), there was no correlation between ER22/23EK polymorphism and early morning cortisol levels. However, we previously reported that the carrier genotype is associated with glucocorticoid resistance, as manifest by a decreased response to the dexamethasone suppression test (2). These data suggest that there may be beneficial metabolic effects, resulting in better survival, due to subtle lifelong glucocorticoid resistance. In the present study, we found that the ER22/23EK polymorphism of the glucocorticoid receptor gene was associated with lower C-reactive protein levels and better survival in elderly men.

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