A study into genes encoding longevity in humans

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CHAPTER 7

Genetic Variants in the Glucocorticoid Receptor Gene (*NR3C1*) and Cardiovascular Disease Risk. The Leiden 85-Plus Study

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Abstract

Recently, the ER22/23EK, N363S and *BclI* polymorphisms in the glucocorticoid receptor (*GR*) gene have been linked to altered cortisol sensitivity and to cortisol-associated disorders. The aim of this study was to investigate the effect of these genetic variants in the *GR* gene on cardiovascular disease and mortality in elderly persons aged 85 years and over. In the population-based Leiden 85-plus Study, 552 participants were genotyped for the ER22/23EK, N363S and *BclI* polymorphisms, and the effects of the polymorphisms on metabolic profile, body composition, and on the prevalence of cardiovascular pathologies at baseline, were assessed. Allcause and cardiovascular disease mortality risks dependent on the SNPs were calculated after a 4.2-year follow-up. The analyses of metabolic profile revealed that carriers of the ER22/23EK polymorphism have higher HbA1C levels ($p<0.001$) and carriers of the N363S SNP have higher LDL cholesterol ($p<0.001$) and triglyceride concentrations ($p=0.03$), compared to the non-carriers. The only signficant association between genotype and body composition analyses was for height and the ER22/23EK polymorphism. Men carrying the ER22/23EK polymorphism were taller ($p=0.02$) compared to non-carriers. No associations with cardiovascular pathologies, allcause and cardiovascular mortality were observed for any of the polymorphisms. We conclude that, in spite the effect of the ER22/23EK and N363S SNPs on metabolism, these polymorphisms together with the BclI SNP, do not affect the risks of cardiovascular disease and survival at old age.

Introduction

In response to various stimuli, including stress, cortisol coordinates metabolic, endocrine, immune, and nervous system responses (Chrousos, 1995; Chrousos, 1998; de Kloet et al., 1998; McEwen, 1998; Munck et al., 1984). Cortisol exerts the majority of its functions through glucocorticoid receptors (GRs). Disturbances in cortisol signaling, resulting either from altered hormone availability or decreased/increased receptor-mediated signal transduction have been associated with a variety of phenotypes including cardiovascular disease, which is a major cause of death at old age.

It is known that sensitivity to cortisol varies considerably between individuals as demonstrated by the dexamethasone suppression test (Huizenga et al., 1998b). Therefore, the underlying susceptibility to cortisol-associated disorders may vary. A degree of inter-individual variation in responsiveness to cortisol is attributable to three polymorphisms in the *GR* gene (NR3C1). Recently an ER22/23EK variation in the *GR* gene was associated with a resistance to cortisol, and shown to result in a better metabolic and cardiovascular health profile, leading to increased survival rate (van Rossum et al., 2002; van Rossum et al., 2004a; van Rossum et al., 2004b). On the other hand, increased sensitivity to cortisol, related to the N363S (Huizenga et al., 1998a) and *BclI* (van Rossum et al., 2003) polymorphisms, has been shown to cause opposite effects. In middle-aged subjects, the N363S and *BclI* polymorphisms were associated with increased body mass index (BMI) and with several risk factors for atherosclerosis and coronary artery disease (Di Blasio et al., 2003; Lin et al., 2003; van Rossum et al., 2003). Together these results suggest that the *GR* gene plays an important role in modulating the susceptibility to cardiovascular disease. Futhermore, there are disparities in the published literature. For instance in some studies, associations between these three polymorphisms and metabolic parameters or body composition have been found (Di Blasio et al., 2003; Huizenga et al., 1998a; Lin et al., 1999; Lin et al., 2003; van Rossum et al., 2002; van Rossum et al., 2003; van Rossum et al., 2004b), whereas in other studies no association, or opposite effects have been observed (Dobson et al., 2001; Echwald et al., 2001; Panarelli et al., 1998; Rosmond et al., 2001; van Rossum et al., 2002; van Rossum et al., 2004a). However, the recent results with cardiovascular disease (Lin et al., 2003) and survival (van Rossum et al., 2004a) have never been replicated in elderly subjects. Therefore, the effect of the GR gene on cardiovascular disease and survival at old age remains to be elucidated.

In this study, we assessed the impact of the ER22/23EK, N363S and *BclI* variants in the *GR* gene on metabolic profile, body composition, and on the prevalence of cardiovascular pathologies. Also the risks of allcause and cardiovascular mortalities dependent on the polymorphisms were calculated. The study was carried out in the population-based Leiden 85-plus Study, using cross-sectional and prospective study designs.

Participants and methods

Participants

The Leiden 85-plus Study is a prospective population based study, in which all 85 year old in-

habitants of Leiden, The Netherlands were invited to take part. There were no selection criteria related to health or demographic characteristics. The study population consists of 599 subjects, all members of the 1912-1914 birth cohort, enrolled in the month of their 85th birthday between 1997 and 1999 (Bootsma-van der Wiel et al., 2002). For the present study DNA was available for 563 people. All participants of the Leiden 85-plus Study were followed for mortality until April 1, 2004. Primary causes of death were obtained from the Dutch Central Bureau of Statistics and categorized according to the 10th International Classification of Diseases (ICD-10). The Medical Ethical Committee of the Leiden University Medical Centre approved the study and informed consent was obtained from all participants. We also genotyped 370 blood donors (median age 32, inerquartile range (27-36)) from Leiden and surrounding areas for a cross-sectional analysis of genotype frequencies (Heijmans et al., 1999).

Metabolic profile and body composition at baseline

C-reactive protein, high-density lipoprotein (HDL)-cholesterol, triglycerides and HbA1c (hemoglobin A1c) concentrations were determined in serum using fully automated analyzers (Hitachi 747 and 911; Hitachi, Ltd, Tokyo, Japan). Low-density lipoprotein (LDL)-cholesterol was estimated with the Friedewald equation (Friedewald et al., 1972). Cortisol was determined by a fluorescence polarization immunoassay using the Abbott TDx (Abbott Laboratories, Abbott Park, IL, USA) according to the manufacturer's instructions. The within-assay coefficient of variation (CV) was below 5 % at different levels. Body weight (kg) and height (cm) were measured in all participants and body mass index (BMI, kg/m^2) was calculated. The measurement of height is often unreliable in elderly, and therefore also armspan (cm) was measured, which approximates to height at maturity.

Cardiovascular pathologies at baseline

The prevalence and number of cardiovascular pathologies were obtained from the participant's general practitioner or nursing home physician. In addition, electrocardiograms were recorded on a Siemens Siccard 440 and transmitted by telephone to the ECG Core Lab in Glasgow for automated Minnesota coding (Macfarlane and Latif, 1996). Cardiovascular pathologies were classified as: myocardial infarction, myocardial ischemia, intermittent claudication or stroke (van Exel et al., 2002).

Genotyping

The ER22/23EK variant consists of two single nucleotide polymorphisms (SNPs) in codons 22 (A/G, rs6189) and 23 (G/A, rs6190). The ER22/23EK, N363S (A/G, rs6195) and *BdI* (C/G) (van Rossum et al., 2003) polymorphisms were genotyped using an Assay-by-Design (Applied Biosystems), consisting of PCR primers and TaqMan MGB probes on an ABI 7900 HT with real-time PCR (Applied Biosystems). Amplification reactions and parameters were based on manufacturer's instructions.

Statistical analysis

Allele frequencies were calculated and analyzed for deviation from the Hardy-Weinberg equilibrium using the χ^2-test. Mean differences in the parameters of the metabolic profile and body composition dependent on the polymorphisms were assessed with the univariate general linear model. All continuous variables were normally distributed, except for the C-reactive protein and triglyceride levels, which were ln-transformed. Differences in the prevalence of cardiovascular pathologies between genotypes were tested using the binary logistic regression model adjusted for sex. Mortality was first estimated using the Kaplan-Meier method, followed by the calculation of sex adjusted mortality risks and 95 % confidence intervals (CI) for allcause and

Table 1. Baseline characteristics of the Leiden 85-plus Study

Number	552
Age	85
Female	366 (66 %)
Independently living	451 (82 %)
Metabolic profile	
Cortisol (mmol/l)	0.49 (0.15)
C-reactive protein (mg/l)	2.83 (4.26)
HDL cholesterol (mmol/l)	1.31 (0.40)
LDL cholesterol (mmol/l)	3.67 (0.97)
Triglycerides (mmol/l)	1.40 (1.58)
HbA1c (mmol/l)	5.79 (1.09)
Body composition	
BMI (kg/m^2)	
Men	26.3 (5.17)
Women	27.7 (4.74)
Weight (kg)	
Men	74.5 (15.2)
Women	67.5 (12.7)
Height (cm)	
Men	168.1 (6.49)
Women	156.2 (6.24)
Armspan (cm)	
Men	178.1 (7.31)
Women	162.5 (7.03)
Cardiovascular pathologies	
Myocardial infarction	134 (24 %)
Myocardial ischemia	280 (51 %)
Intermittent claudication	34 (6 %)
Stroke	56 (10 %)

Metabolic profile and body composition values presented as means (SD), except for C-reactive protein where geometric mean (SD) is presented

cardiovascular mortality with the Cox proportional hazard model. All analyses were performed with SPSS statistical software, version 12.0 (Chicago, Illinois, USA), with the exception of the mortality analyses, which were performed with STATA software, version 9.0 (Texas, USA).

Results

DNA was available for 563 participants, of which 11 were excluded due to the use of corticosteroids. The baseline characteristics of the remaining 552 participants are presented in Table 1. All study subjects were genotyped for the ER22/23EK, N363S and *BclI* polymorphisms with a genotyping error of lower than 5 %. Therefore the total number of analyzed participants was 540 for ER22/23EK, 548 for N363S and 526 for the *BclI* polymorphisms. The minor allele frequencies were 0.03, 0.05 and 0.34 for the ER22/23EK, N363S and *BclI* polymorphisms respectively. The overall genotype distributions and resulting allelic frequencies of the SNPs were in agreement with the distribution predicted by the Hardy-Weinberg equilibrium. In this study, the calculated allele and genotype frequencies did not differ between elderly and young subjects (n=370, median age 32 years)(data not shown).

At baseline, several parameters of the metabolic profile dependent on the ER22/23EK, N363S and *BclI* variants were assessed. The analyses revealed that carriers of the ER22/23EK variant have higher HbA1c levels (6.54 (0.19) vs. 5.74 (0.05), $p<0.001$) compared to the non-carriers (Table 2). For the carriers of the N363S polymorphism, higher concentrations of LDL cholesterol (4.18 (0.13) vs. 3.62 (0.04), $p<0.001$) and triglycerides (1.60 (1.06) vs. 1.39 (1.02), $p=0.03$) compared to the non-carriers were observed (Table 2). No associations between the metabolic profile and the *BclI* polymorphism were found. The analyses of body composition showed only differences in height for the ER22/23EK polymorphism, where men carrying the ER22/23EK variant were taller (171.9 (1.71) vs. 167.8 (0.50), $p=0.02$) compared to the non-carriers. The same trend was observed for women (Table 3).

The effect of the polymorphisms on the prevalence of cardiovascular pathologies was assessed at baseline. No associations with the prevalence of myocardial infarction, myocardial ischemia, intermittent claudication and stroke were found for the different polymorphisms (Table 4).

During the 4.2-year follow-up, 278 (50 %) of the participants had died, of which 115 (41 %) of deaths were due to cardiovascular disease. The Kaplan-Meier estimates of cumulative mortality indicated that the carriers of *BclI* polymorphism have lower allcause mortality during the follow-up compared to the non-carriers (Figure 1). The all-cause mortality risk estimate for the *BclI* variant was 0.85 (0.67-1.08). The cardiovascular mortality analyses revealed no differences in survival for any of the ER22/23EK, N363S and *BclI* polymorphism carriers (Figure 1). The results remained unaltered after repeating the analyses for men and women separately.

All the above mentioned analyses were also repeated with independently living participants (n=451), in order to implement the selection criteria used in other studies (van Rossum et al., 2003; van Rossum et al., 2004a). These analyses did not reveal additional associations, but confirmed those observed in the whole cohort.

Table 2. Metabolic profile and body composition dependent on the ER22/23EK (G/A), N363S (A/G) and *BclI* (C/G) polymorphisms

	ER22/23EK			N363S			*BslI* C/G		
	GG (n=507) mean (SE)	GA/AA (n=33) mean (SE)	p-value	AA (n=497) mean (SE)	AG/GG (n=51) mean (SE)	p-value	CC (n=236) mean (SE)	CG/GG (n=290) mean (SE)	p-value
Cortisol (mmol/l)	0.49 (0.01)	0.49 (0.03)	0.90	0.49 (0.01)	0.51 (0.02)	0.35	0.48 (0.01)	0.50 (0.01)	0.10
C-reactive protein (mg/l)	2.80 (1.06)	3.35 (1.30)	0.51	2.86 (1.07)	2.80 (1.22)	0.91	2.69 (1.09)	3.06 (1.09)	0.29
HDL cholesterol (mmol/l)	1.31 (0.02)	1.26 (0.07)	0.45	1.31 (0.02)	1.26 (0.05)	0.33	1.31 (0.03)	1.32 (0.02)	0.87
LDL cholesterol (mmol/l)	3.66 (0.04)	3.67 (0.17)	0.92	3.62 (0.04)	4.18 (0.13)	<0.001	3.72 (0.06)	3.63 (0.06)	0.29
Triglycerides (mmol/l)	1.40 (1.02)	1.32 (1.08)	0.47	1.39 (1.02)	1.60 (1.06)	0.03	1.40 (1.03)	1.39 (1.03)	0.81
HbA1c (mmol/l)	5.74 (0.05)	6.54 (0.19)	<0.001	5.81 (0.05)	5.72 (0.15)	0.60	5.78 (0.07)	5.80 (0.06)	0.84

Sex adjusted univariate general linear model. For C-reactive protein and triglycerides geometric means with standard errors (SE) are presented

Table 3. Body composition dependent on the ER22/23EK (G/A), N363S (A/G) and *BclI* (C/G) polymorphisms

	ER22/23EK			N363S			*BslI* C/G		
	GG (n=507) mean (SE)	GA/AA (n=33) mean (SE)	p-value	AA (n=497) mean (SE)	AG/GG (n=51) mean (SE)	p-value	CC (n=236) mean (SE)	CG/GG (n=290) mean (SE)	p-value
BMI (kg/m^2)									
Men	26.3 (0.42)	26.1 (1.40)	0.91	26.3 (0.41)	26.0 (1.39)	0.84	25.8 (0.59)	26.6 (0.54)	0.30
Women	27.8 (0.26)	27.7 (1.15)	0.99	27.7 (0.27)	27.5 (0.80)	0.77	27.2 (0.39)	28.1 (0.35)	0.06
Weight (kg)									
Men	74.3 (1.22)	77.9 (4.12)	0.40	74.7 (1.19)	72.8 (4.09)	0.65	73.4 (1.72)	75.2 (1.59)	0.43
Women	67.4 (0.70)	69.6 (3.07)	0.48	67.5 (0.72)	67.2 (2.11)	0.90	66.4 (1.05)	68.2 (0.94)	0.19
Height (cm)									
Men	167.8 (0.50)	171.9 (1.71)	0.02	168.3 (0.50)	167.2 (1.73)	0.56	168.4 (0.69)	167.8 (0.65)	0.48
Women	156.0 (0.34)	158.6 (1.47)	0.09	156.2 (0.35)	156.6 (1.04)	0.73	156.3 (0.52)	156.1 (0.46)	0.73
Armspan (cm)									
Men	177.8 (0.57)	180.6 (1.97)	0.18	178.3 (0.56)	176.5 (1.93)	0.36	178.7 (0.79)	177.6 (0.74)	0.31
Women	162.4 (0.39)	164.3 (1.66)	0.26	162.5 (0.40)	162.6 (1.17)	0.91	162.5 (0.58)	162.4 (0.52)	0.85

Univariate general linear model. Data presented as means with standard errors (SE)

Table 4. Risk of cardiovascular pathologies at baseline dependent on the ER22/23EK (G/A), N363S (A/G) and *BclI* (C/G) polymorphisms

	ER22/23EK		N363S		*BclI*	
	GG n=507	GA/AA n=33	AA n=497	AG/GG n=51	CC n=236	CG /GG n=290
Myocardial infarction (n=134)	1	1.35 (0.62-2.93)	1	1.25 (0.65-2.40)	1	0.91 (0.61-1.37)
Myocardial ischemia (n=280)	1	1.82 (0.87-3.78)	1	1.41 (0.78-2.52)	1	0.80 (0.57-1.13)
Intermittent claudication (n=34)	1	0.87 (0.20-3.86)	1	1.03 (0.30-3.52)	1	1.00 (0.49-2.04)
Stroke (n=56)	1	1.56 (0.58-4.24)	1	1.79 (0.79-4.05)	1	1.22 (0.69-2.16)

Sex adjusted binary logistic regression. Data presented as odds ratios (OR) with 95 % confidence intervals (CI)

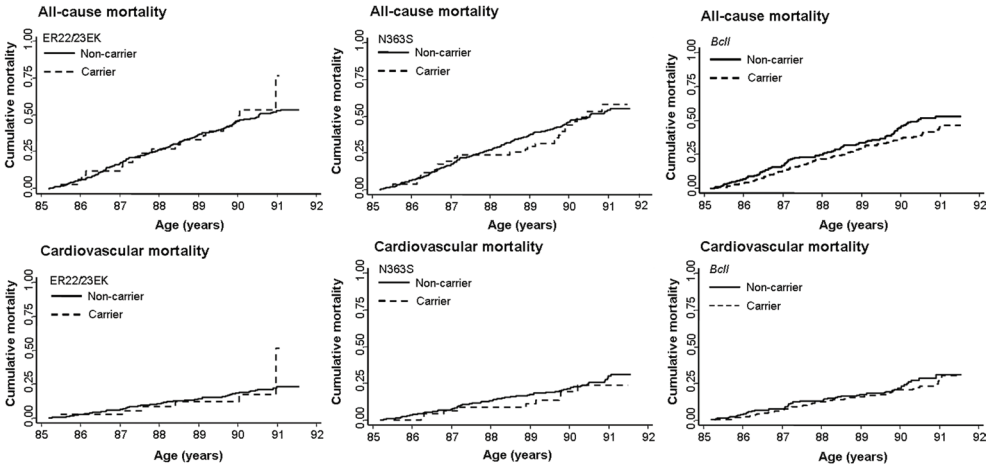

Figure 1. Kaplan-Meier curves of cumulative all-cause and cardiovascular mortality, dependent on the ER22/23EK, N363S and *BclI* genotypes in the 552 participants from age 85 years onwards.

Discussion

The aim of this study was to examine whether in old age polymorphisms in the GR gene (NR3C1) influence the prevalence of cardiovascular pathologies and survival due to differences in metabolic profile and body composition. The analyses revealed differences in metabolic profile for the ER22/23EK and N363S SNPs, and in body composition for the ER22/23EK variant but no associations with the prevalence of cardiovascular pathologies and lifespan.

Increased sensitivity to cortisol, accompanied with sub-optimal metabolic profile and increased risk for cardiovascular disease has been associated with the N363S and *BclI* polymorphisms (Di Blasio et al., 2003; Lin et al., 2003; van Rossum et al., 2003). In this study, we found no associations with the *BclI* polymorphism, but carriers of the N363S SNP had higher LDL-cholesterol and triglyceride levels, and a non-significantly higher prevalence for cardiovascular

diseases. The latter results are in accordance with another study in middle aged subjects (Lin et al., 2003), where N363S variant was associated with elevated cholesterol and triglyceride concentrations, and also with coronary artery disease. The lack of a relation between the N363S polymorphism and cardiovascular pathologies in our study population could be due to the fact that in the elderly high levels of LDL-cholesterol and triglycerides are not risk factors for cardiovascular disease anymore (Weverling-Rijnsburger et al., 1997; Weverling-Rijnsburger et al., 2003).

For the ER22/23EK variant, associations opposite of those expected were observed. It has been previously reported that elderly carriers of the ER22/23EK polymorphism have lower total cholesterol and LDL-cholesterol levels, lower fasting insulin concentrations, better insulin sensitivity and lower C-reactive protein levels, leading to a beneficial metabolic profile (van Rossum et al., 2002; van Rossum et al., 2004a). In this study, carriers of the ER22/23EK variant had higher levels of HbA1c compared to non-carriers, and a trend for higher C-reactive protein and LDL-cholesterol concentrations, which indicate a worse metabolic profile. Furthermore, no beneficial effects of the ER22/23EK polymorphism on the prevalence of cardiovascular pathologies, all-cause and cardiovascular mortality were found. These result are at odds with results from the previously published studies (van Rossum et al., 2002; van Rossum et al., 2004a). It could be, that the discrepancy between these studies has arisen due to the age difference of the subjects. In our study, participants aged 85-90 years were analyzed compared to subjects aged 67-77 years in the other studies (van Rossum et al., 2002; van Rossum et al., 2004a). The analyzed polymorphisms might have an effect before a certain age, and therefore the Leiden 85-plus cohort could consists of survivors. To test this, allele and genotype frequencies between the elderly and young participants in this study, and also between a young study group from the published literature (van Rossum et al., 2004b) were compared, revealing no differences. Thus, in contrast to an earlier report of an age-dependent enrichment for the ER22/23EK variation (van Rossum et al., 2002), no such enrichment was observed in our study.

Another possible source for differences could come from the participant's inclusion criteria. In order to apply similar inclusion criteria used in the other studies, only independently living participants were analyzed. However, this did not change our results, as well as stratification for gender. A more likely explanation could be that some of the observations have arisen by chance, leading to false negative or false positive associations. Recently it has been suggested that the role of genetic variations in common traits should be built up based on the evidence of many studies. Significant between-study diversity is frequent, and the results of the first study often correlate only modestly with subsequent research on the same association (Ioannidis et al., 2001).

The results of this study raise doubts whether the analyzed polymorphisms, shown to result in either resistance or increased sensitivity to cortisol (Huizenga et al., 1998a; van Rossum et al., 2002; van Rossum et al., 2003), are really functional. For the ER22/23EK variant, it has been reported that it does not alter the *in vitro* capacity to activate transcription (de Lange et al., 1997) and no altered response to cortisol was observed (Koper et al., 1997). However, a more recent study shows, that the transcriptional activity of the ER22/23EK carriers is decreased, due to higher expression of less transcriptionally active GR-A protein in the ER22/23EK carriers. It was proposed that this leads to the decrease in the sensitivity to cortisol (Russcher et al., 2005). For the other two polymorphisms, N363S and *BclI*, the functionality has not been established *in vitro*. On the other hand, the polymorphisms could be functional and result in altered hormone

sensitivity, which is counterbalanced by adaptive changes that attempt to normalize cortisol signaling. These adaptive changes may vary between individuals.

The strengths of this study are the ability to estimate several phenotypes in one cohort, and the prospective analysis with a high number of deaths, including the high number of cardiovascular deaths, during the follow-up. A weakness of the study is the lack of data on HPA-axis reactivity or on other parameters reflecting the stress system activity. However, in this aspect, we rely on the information presented in the published literature.

In conclusion, the results of this study show that in old age the ER22/23EK and N363S polymorphisms but not the *BclI* SNP in the *GR* gene have an influence on metabolic profile. However, the noticed differences in metabolic profile do not affect the prevalence of cardiovascular pathologies and the risks for all-cause and cardiovascular disease mortality at old age.

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References

Bootsma-van der Wiel A, van Exel E, De Craen AJ, Gussekloo J, Lagaay AM, Knook DL et al (2002). A high response is not essential to prevent selection bias: results from the Leiden 85-plus study. J Clin Epidemiol 55: 1119-1125.

Chrousos GP (1995). The Hypothalamic-Pituitary-Adrenal Axis and Immune-Mediated Inflammation. N Engl J Med 332: 1351-1363.

Chrousos GP (1998). Stressors, Stress, and Neuroendocrine Integration of the Adaptive Response: The 1997 Hans Selye Memorial Lecture. Ann NY Acad Sci 851: 311-335.

de Kloet ER, Vreugdenhil E, Oitzl MS, Joels M (1998). Brain corticosteroid receptor balance in health and disease. Endocr Rev 19: 269-301.

de Lange P, Koper JW, Huizenga NA, Brinkmann AO, de Jong FH, Karl M et al (1997). Differential hormone-dependent transcriptional activation and -repression by naturally occurring human glucocorticoid receptor variants. Mol Endocrinol 11: 1156-1164.

Di Blasio AM, van Rossum EF, Maestrini S, Berselli ME, Tagliaferri M, Podesta F et al (2003). The relation between two polymorphisms in the glucocorticoid receptor gene and body mass index, blood pressure and cholesterol in obese patients. Clin Endocrinol (Oxf) 59: 68-74.

Dobson MG, Redfern CP, Unwin N, Weaver JU (2001). The N363S polymorphism of the glucocorticoid receptor: potential contribution to central obesity in men and lack of association with other risk factors for coronary heart disease and diabetes mellitus. J Clin Endocrinol Metab 86: 2270-2274.

Echwald SM, Sorensen TI, Andersen T, Pedersen O (2001). The Asn363Ser variant of the glucocorticoid receptor gene is not associated with obesity or weight gain in Danish men. Int J Obes Relat Metab Disord 25: 1563-1565.

Friedewald WT, Levy RI, Fredrickson DS (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499-502.

Heijmans BT, Gussekloo J, Kluft C, Droog S, Lagaay AM, Knook DL et al (1999). Mortality risk in men is associated with a common mutation in the methylene-tetrahydrofolate reductase gene (MTHFR). Eur J Hum Genet 7: 197-204.

Huizenga NA, Koper JW, de Lange P, Pols HA, Stolk RP, Burger H et al (1998a). A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids in vivo. J Clin Endocrinol Metab 83: 144-151.

Huizenga NA, Koper JW, de Lange P, Pols HA, Stolk RP, Grobbee DE et al (1998b). Interperson variability but intraperson stability of baseline plasma cortisol concentrations, and its relation to feedback sensitivity of the hypothalamo-pituitary-adrenal axis to a low dose of dexamethasone in elderly individuals. J Clin Endocrinol Metab 83: 47-54.

Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG (2001). Replication validity of genetic association studies. Nat Genet 29: 306-309.

Koper JW, Stolk RP, de Lange P, Huizenga NA, Molijn GJ, Pols HA et al (1997). Lack of association between five polymorphisms in the human glucocorticoid receptor gene and glucocorticoid resistance. Hum Genet 99: 663-668.

Lin RC, Wang WY, Morris BJ (1999). High penetrance, overweight, and glucocorticoid receptor variant: case-control study. BMJ %20;319: 1337-1338.

Lin RC, Wang XL, Morris BJ (2003). Association of coronary artery disease with glucocorticoid recep-

tor N363S variant. Hypertension 41: 404-407.

Macfarlane PW, Latif S (1996). Automated serial ECG comparison based on the Minnesota code. J Electrocardiol 29 Suppl: 29-34.

McEwen BS (1998). Protective and Damaging Effects of Stress Mediators. N Engl J Med 338: 171-179.

Munck A, Guyre PM, Holbrook NJ (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr Rev 5: 25-44.

Panarelli M, Holloway CD, Fraser R, Connell JM, Ingram MC, Anderson NH et al (1998). Glucocorticoid receptor polymorphism, skin vasoconstriction, and other metabolic intermediate phenotypes in normal human subjects. J Clin Endocrinol Metab 83: 1846-1852.

Rosmond R, Bouchard C, Bjorntorp P (2001). Tsp509I polymorphism in exon 2 of the glucocorticoid receptor gene in relation to obesity and cortisol secretion: cohort study. BMJ 322: 652-653.

Russcher H, van Rossum EF, de Jong FH, Brinkmann AO, Lamberts SW, Koper JW (2005). Increased Expression of the Glucocorticoid Receptor-A Translational Isoform as a Result of the ER22/23EK Polymorphism. Mol Endocrinol 19: 1687-1696.

van Exel E, Gussekloo J, Houx P, de Craen AJM, Macfarlane PW, Bootsma-van der Wiel A et al (2002). Atherosclerosis and cognitive impairment are linked in the elderly. The Leiden 85-plus Study. Atherosclerosis 165: 353-359.

van Rossum EF, Feelders RA, Van Den Beld AW, Uitterlinden AG, Janssen JA, Ester W et al (2004a). Association of the ER22/23EK polymorphism in the glucocorticoid receptor gene with survival and C-reactive protein levels in elderly men. Am J Med 117: 158-162.

van Rossum EF, Koper JW, Huizenga NA, Uitterlinden AG, Janssen JA, Brinkmann AO et al (2002). A polymorphism in the glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids in vivo, is associated with low insulin and cholesterol levels. Diabetes 51: 3128-3134.

van Rossum EF, Koper JW, Van Den Beld AW, Uitterlinden AG, Arp P, Ester W et al (2003). Identification of the BclI polymorphism in the glucocorticoid receptor gene: association with sensitivity to glucocorticoids in vivo and body mass index. Clin Endocrinol (Oxf) 59: 585-592.

van Rossum EF, Voorhoeve PG, te Velde SJ, Koper JW, Delemarre-van de Waal HA, Kemper HC et al (2004b). The ER22/23EK polymorphism in the glucocorticoid receptor gene is associated with a beneficial body composition and muscle strength in young adults. J Clin Endocrinol Metab 89: 4004-4009.

Weverling-Rijnsburger AW, Blauw GJ, Lagaay AM, Knook DL, Meinders AE, Westendorp RG (1997). Total cholesterol and risk of mortality in the oldest old. Lancet 350: 1119-1123.

Weverling-Rijnsburger AW, Jonkers IJ, van Exel E, Gussekloo J, Westendorp RG (2003). High-density vs low-density lipoprotein cholesterol as the risk factor for coronary artery disease and stroke in old age. Arch Intern Med 163: 1549-1554.