Glucocorticoid Receptor Haplotype and Metabolic Syndrome: the Lifelines Cohort Study

Wester VL, Koper JW, van den Akker ELT, Franco OH, Stolk RP, van Rossum EFC.

Eur J Endocrinol 2016; 175: 645-651.

ABSTRACT

Objective: An excess of glucocorticoids (Cushing's syndrome) is associated with metabolic syndrome (MetS) features. Several single nucleotide polymorphisms (SNPs) in the glucocorticoid receptor (GR) gene influence sensitivity to glucocorticoids, and have been associated with aspects of MetS. However, results are inconsistent, perhaps due to the heterogeneity of the studied populations and limited samples. Furthermore, the possible association between functional GR SNPs and prevalence of MetS remains unexplored.

Design: Cross-sectional population based cohort study.

Methods: MetS presence and carriage of functional GR SNPs (BclI, N363S, ER22/23EK, GR-9beta) were determined in 12,552 adult participants from Lifelines, a population-based cohort study in the Netherlands. GR SNPs were used to construct GR haplotypes.

Results: Five haplotypes accounted for 99.9% of all GR haplotypes found. No main effects of functional GR haplotypes on MetS were found, but the association of GR haplotype 4 (containing N363S) with MetS was influenced by interaction with age, sex and education status ($P<0.05$). Stratified analysis revealed that haplotype 4 increased MetS presence in younger men (at or below the median age of 47; odds ratio 1.77, $P=0.005$) and in people of low education status (odds ratio 1.48, $P=0.039$).

Conclusions: A glucocorticoid receptor haplotype, which confers increased sensitivity to glucocorticoids, appears to increase the risk of metabolic syndrome, but only among younger men and lower educated individuals, suggesting gene-environment interactions.

INTRODUCTION

The Metabolic Syndrome (MetS) is a cluster of cardiometabolic risk factors associated with cardiovascular disease, diabetes and increased mortality [1, 2]. An individual is considered to have MetS if three out of the following five risk factors are present: abdominal obesity, hypertension, high triglycerides, low HDL cholesterol and insulin resistance [1]. Approximately one fourth of adults in Europe is reported to fulfil criteria for MetS [3], and MetS is known to double the risk of incident cardiovascular disease [2].

Glucocorticoid hormones, of which cortisol is the most important in humans, affect metabolism, behavior and circulation [4]. An excess of glucocorticoids, which occurs in endogenous hypercortisolism as well as due to glucocorticoid administration, results in Cushing's syndrome (CS) [5]. CS commonly leads to central obesity and MetS features [5]. Furthermore, slight increases in long-term cortisol exposure have been linked to increases in MetS presence, cardiovascular disease and obesity [6, 7].

The tissue effects of glucocorticoids are influenced by glucocorticoid sensitivity, which is partly determined by functional single-nucleotide polymorphisms (SNPs) in the glucocorticoid receptor (GR) gene. These polymorphisms are associated with cardiometabolic risk factors in an age-dependent manner [4, 8]. The *Bcl*I polymorphism increases glucocorticoid sensitivity *in vivo* as assessed using dexamethasone suppression testing [9], and has been associated with increased BMI, central adiposity and insulin resistance [10, 11]. The N363S polymorphism increases the *in vivo* sensitivity to glucocorticoids, as well as the transactivational capacity of the GR [12-14] which is presumed to be responsible for most of the metabolic effects of glucocorticoids [15]. In line with this, N36S carriage has been associated with increased BMI, and higher LDL cholesterol in the elderly [16, 17]. In contrast, the ER22/23EK polymorphism has been shown to decrease GR transactivation [13]. ER22/23EK has been associated with a beneficial metabolic profile with subtle increases in lean mass in younger individuals, and increased insulin sensitivity, lower LDL cholesterol and decreased cerebrovascular pathology in the elderly [18-20]. We also previously showed that the GR-9beta polymorphism does not affect GR transactivation, but reduces the transrepression activity of the GR, and thereby the immunosuppressive actions of glucocorticoids [16, 21]. Carriage of GR-9beta has been associated with an increased incidence of coronary heart disease [22]. These functional GR polymorphisms show a high level of linkage disequilibrium, and can be integrated to distinguish five different haplotypes of the GR gene [23].

To the best of our knowledge, no study has investigated the associations between GR haplotypes and the presence of MetS. Previous studies indicate that variations in the

GR may affect the cardiometabolic profile in an age-dependent manner. Therefore, we aimed to study the associations between GR haplotypes and MetS in a large population based cohort, providing an opportunity to investigate how this association may vary in subgroups with respect to age, sex and socioeconomic status.

SUBJECTS AND METHODS

Participants and procedures

Lifelines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviors of 167,729 persons living in the north of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics [24]. Lifelines is a facility that is open for all researchers. Information on the application and data access procedure is summarized on www.lifelines.net. Detailed information on all collected variables can be found in the online Lifelines Data Catalogue (https://catalogue.lifelines.nl/).

Each participant was invited for a site visit, which included a fasting venipuncture and a physical examination including measurement of blood pressure, weight, height, and waist circumference. In addition, all participants were asked to fill out a set of questionnaires, including an extensive medical history. We included all adult participants in whom GWAS (genome wide association study) and cardiometabolic data were available (n=12,552). This study was approved by the Medical Ethics Review Committee of the University Medical Center Groningen. All study procedures were carried out in accordance with the declaration of Helsinki, and all participants provided written informed consent.

Laboratory measurements and MetS diagnosis

In each participant, blood was drawn after an overnight fast. Glucose, triglycerides and HDL cholesterol were determined using routine laboratory procedures. MetS presence was defined according to the commonly used NCEP ATPIII criteria [25], when participants fulfilled at least three of the following five criteria: abdominal obesity (waist circumference $\geq$102 cm in men, $\geq$88 cm in women), elevated triglycerides (serum triglycerides $\geq$1.7 mmol/L and/or use of lipid-lowering medication), reduced HDL cholesterol (serum HDL <1.0 mmol/L in men and <1.3 nmol/L in women, and/or use of lipid-lowering medication), elevated blood pressure (systolic $\geq$130 and/or diastolic $\geq$85 mmHg and/or use of

antihypertensives) and elevated fasting glucose (fasting plasma glucose ≥5.6 mmol/L, and/or use of blood glucose lowering medication, and/or diagnosis of type 2 diabetes mellitus). In addition to the ATPIII criteria, we used MetS criteria of the International Diabetes Federation (IDF) in sensitivity analyses, in order to examine the robustness of our findings [26].

Genomic analysis

Genotyping was performed using the Illumina HumanCytoSNP GWAS platform. The GWAS set was enriched using imputation with 1000 genomes as a reference set. From this enriched GWAS dataset, genotypes of functional GR SNPs (ER22/23EK, rs6189 and rs6190; N363S rs56149945; *Bcl*I, rs41423247; GR-9β, rs6198) were extracted using PLINK version 1.08p (Shaun Purcell, Harvard University) [27]. PHASE version 2.1 (Matthew Stephens Lab, University of Chicago) was used to construct GR haplotypes based on these SNPs [28, 29].

Statistical analysis

SPSS version 21 was used for descriptive statistics, and R statistics (version 3.2.1) was used for regression analysis. The association between GR haplotype status (carriers vs. non-carriers) and MetS was studied using logistic regression. Analyses were adjusted for covariates with a significant influence on MetS presence ($P<0.05$), which included smoking status, education level, age and sex. Smoking status was defined as no smoking, past smoking for at least one year, and current smoking. Education level was stratified in three categories of attained education levels: low, middle or high. In order to adjust the models for smoking and education status, dummy variables were created for low education status, high education status, past smoking and current smoking. In order to study interaction effects between covariates and GR haplotypes, we studied whether addition of an interaction term (haplotype*covariate) significantly changed the model, using the likelihood ratio test. Significant interactions were explored using stratified analysis with Bonferroni adjustment for multiple testing. *P*-values of <0.05 were considered to indicate statistical significance.

RESULTS

Baseline characteristics are described in Table 1. In the total study population MetS prevalence was 21.9%, and was higher in men than in women (25.1 vs 19.6%, P<0.001). Five haplotypes accounted for over 99.9 percent of all GR haplotypes, the frequencies of which are depicted in Figure 1. Haplotype frequencies were similar to the frequencies we reported in another large ethnically similar cohort in the Netherlands [22].

Table 1. baseline characteristics

	Females N=7327	Males N=5225	P value
Age (years), median (IQR)	47 (41 - 55)	48 (41 - 57)	0.025
Smoking status (%)			<0.001
Current	23.8	27.8	
Past	35.2	38.7	
Education level (%)			<0.001
Lower	19.6	22.9	
Middle	55.2	46.5	
Higher	22.8	29.1	
Cardiometabolic parameters, median (IQR)			
Body mass index (kg/m2)	25.4 (22.9 - 28.5)	26.3 (24.3 - 28.6)	<0.001
Waist cirumference (cm)	88 (80 - 96)	96 (90 - 103)	<0.001
Triglycerides (mmol/L)	0.93 (0.70 - 1.31)	1.21 (0.88 - 1.75)	<0.001
HDL cholesterol (mmol/L)	1.5 (1.3 - 1.8)	1.2 (1.0 - 1.4)	<0.001
Glucose (mmol/L)	4.9 (4.6 - 5.2)	5.1 (4.8 - 5.5)	<0.001
Systolic blood pressure (mmHg)	123 (114 - 134)	132 (123 - 142)	<0.001
Metabolic syndrome (%)	19.6	25.1	<0.001

Abbreviations: IQR, interquartile range; HDL, high density lipoprotein.
Differences between females and males were tested using Mann-Whitney U tests (continuous variables) or Chi square tests (categorical variables).

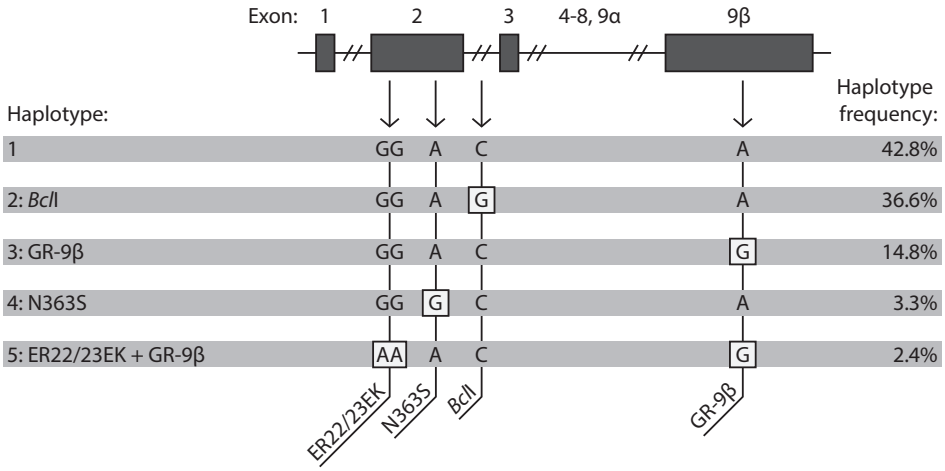

Figure 1. glucocorticoid receptor haplotype frequencies

Effect of GR haplotypes on MetS presence

Logistic regression analysis showed no effects for the functional GR receptor haplotypes on MetS presence (P>0.05, Table 2). A sensitivity analysis using the IDF criteria for MetS

did not change this result (data not shown). For GR haplotype 4, which contains the N363S polymorphism, there was a significant interaction between haplotype carrier status and sex (χ^2(df=1)=4.22, P=0.040), age (χ^2(df=1)=9.70, P=0.002), and education (χ^2(df=2)=8.51, P=0.014), therefore we performed stratified analysis to explore these associations.

Table 2. association between functional glucocorticoid receptor haplotype carrier status (1/2 copies vs. 0 copies) and metabolic syndrome

	Simple model		Adjusted model[a]	
	OR (95%CI)	P value	OR (95%CI)	P value
Haplotype 2: *Bcl*I	0.93 (0.85 - 1.02)	0.107	0.93 (0.85 - 1.02)	0.106
Haplotype 3: GR-9beta	1.03 (0.94 - 1.14)	0.474	1.05 (0.95 - 1.15)	0.371
Haplotype 4: N363S	1.03 (0.87 - 1.22)	0.755	1.01 (0.85 - 1.21)	0.894
Haplotype 5: ER22/23EK + GR-9beta	0.90 (0.73 - 1.11)	0.317	0.91 (0.73 - 1.12)	0.374

Abbreviations: OR, odds ratio; CI, confidence interval.
[a]Adjusted for age, sex, education level, and smoking status.

Stratified analysis for the effect of haplotype 4

After stratification of our study population into two age groups, carriers of haplotype 4 at or below the median age (47 years) had a higher MetS presence (19.9 vs. 14.3%, adjusted odds ratio [OR] 1.46 [95%CI 1.08 – 1.95], P=0.008; Table 3). Further analyses showed no difference within age-strata under the median, or within strata above the median of the distribution, indicating a real difference around 47 years (data not shown). Stratification for both age and sex showed that this haplotype 4 dependent increase at younger age was particularly pronounced in men (28.1 vs. 18.0%, OR 1.77 [95%CI 1.14 – 2.77], P=0.005; Figure 2, Table 3). After stratification for sex alone, no effect of haplotype 4 on MetS was observed in females and males separately (P>0.05; Table 3). Haplotype 4 significantly increased the odds for MetS in individuals with a low educations status (39.9 vs. 31.9%, OR 1.47 [95% confidence interval (CI) 1.08 – 2.01], P=0.015; Figure 2, Table 3), but not in individuals with middle or high education status (P>0.05). Haplotype 4 frequencies were not significantly different between the strata for education status, age, sex, or age and sex (Chi square tests, all P values >0.2). Sensitivity analyses using the IDF criteria for MetS showed that haplotype 4 selectively increased MetS presence in individuals at or below the median age of 47 (OR 1.41 [95%CI 1.07 – 1.86], P=0.010), and in men at or below the age of 47 (OR 1.68 [95%CI 1.11 – 2.55], P=0.007). Unlike our findings when used ATPIII criteria, haplotype 4 did not significantly increase the presence of IDF-MetS in low educated individuals (OR 1.27 [95%CI 0.87 – 1.85], P=0.375).

Table 3. results of stratified analysis for the association between glucocorticoid receptor haplotype 4 carrier status (1/2 copies vs. 0 copies) and metabolic syndrome

		Haplotype 4 effect	
Subgroup	Number of participants	OR(95%CI)	P value
Education status			
Low	2614	**1.48 (1.01 - 2.17)**[a]	**0.039**
Middle	6427	0.83 (0.60 - 1.15)[a]	0.497
High	3165	0.84 (0.49 - 1.44)[a]	1.000
Age			
≤47	6212	**1.46 (1.08 - 1.95)**[b]	**0.008**
>47	6340	0.77 (0.59 - 1.01)[b]	0.065
Sex			
Female	7327	0.84 (0.63 - 1.12)[c]	0.352
Male	5225	1.22 (0.92 - 1.62)[c]	0.229
Age and sex			
Female ≤47	3681	1.18 (0.72 - 1.93)[c]	1.000
Female >47	3646	0.68 (0.45 - 1.04)[c]	0.096
Male ≤47	2531	**1.77 (1.14 - 2.77)**[c]	**0.005**
Male >47	2694	0.88 (0.57 - 1.37)[c]	1.000

Abbreviations: OR, odds ratio; CI, confidence interval.
[a]Adjusted for age, sex, and smoking status.
[b]Adjusted for age, sex, education level, and smoking status.
[c]Adjusted for age, education level, and smoking status.

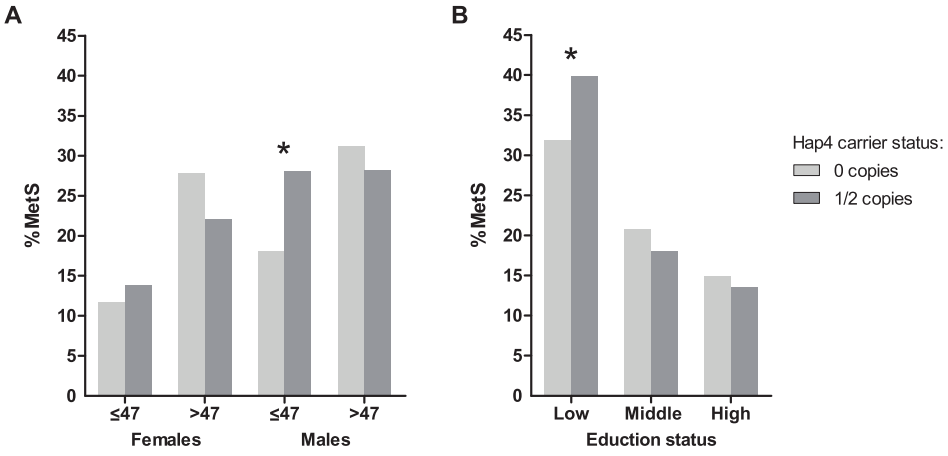

Figure 2. metabolic syndrome presence according to haplotype 4 (N363S) carriage, stratified by sex and age quantiles (A) and education status (B)
Abbreviations: Hap4, glucocorticoid receptor haplotype 4; MetS, metabolic syndrome.
*P-value <0.05. P values in Panel A are adjusted for age, education level, and smoking status. P values in Panel B are adjusted for age, sex, and smoking status.

DISCUSSION

In this study, a glucocorticoid receptor haplotype (marked by the N363S polymorphism), which is known to increase glucocorticoid sensitivity, increased the risk of MetS, but only in specific subgroups of our study population. Although no main effect was observed when the entire cohort was analyzed, a selective increase was seen in younger males, and in people of low education status. Our finding that haplotype 4 increases MetS prevalence specifically in low educated individuals might indicate a gene-environment interaction, in which a genetic constitution consisting of increased glucocorticoid sensitivity only results in an adverse cardiometabolic risk profile against a background of lower educational levels. One explanation for this observation could be that low education status, indicative of lower socioeconomic status, is associated with lifestyle factors that promote MetS [30], which may potentiate the metabolic consequences of increased glucocorticoid sensitivity. Alternatively, socioecomic status might be related to altered glucocorticoid signaling. Recently, we reported an association between low socioeconomic status and increased long-term cortisol levels in children [31]. Although in the present study we only examined adults, a chronic increase in cortisol levels would be expected to augment the metabolic effects of increased glucocorticoid sensitivity. Our study is limited by the fact that we did not have information about cortisol levels.

In vitro studies have shown that the polymorphism N363S, which marks GR haplotype 4, is associated with increased transactivational activity of the GR [13, 14]. This is in line with increased MetS presence, since transactivation is thought to be responsible for metabolic effects of glucocorticoids [15], which include dyslipidemia, insulin resistance and central adiposity [5].

A major strength of the present study is that for the first time, we were able to study the effects of GR haplotypes on MetS presence in a large, well-phenotyped population-based cohort study. Previously, it has been shown that the participants in this cohort form a good representation of inhabitants of the north of the Netherlands [32]. This also means that the Lifelines cohort consists almost exclusively of indigenous Dutch, which limits the extent to which our results can be extrapolated to other ethnic populations. In our study we chose to focus on MetS presence, and not on associations with mean levels of HDL cholesterol, triglycerides, blood pressure and adiposity. Because MetS presence has a clear prognostic value [2], this allowed us to examine the clinical relevance of GR haplotypes.

Earlier studies investigating the associations between GR variations and the cardio-metabolic phenotype were substantially smaller, and often included a range of differ-

ent metabolic outcomes [10, 11, 16-20]. Both small sample size and a high number of statistical tests increase the risk of type I error (i.e. false positives). Although no study design can completely eliminate this risk, we took several measures to reduce it. First, we only used one outcome measure: MetS presence. Second, we limited the number of secondary analyses performed, by using a stepwise approach where stratified analyses were only performed when a genotype-phenotype interaction significantly improved the model. Thirdly, we used multiple testing adjustment to curtail the risk of type I error in our stratified analyses.

Unlike genome wide association studies, we only studied functional variations in the GR gene, using a hypothesis driven approach. Our study design was based on both in vitro and clinical research, and we therefore only included variants with a known influence on glucocorticoid signaling. Based on results obtained from dexamethasone suppression tests [9, 18] and for haplotype 5 also from functional *in vitro* studies [13], we expected an increased prevalence of MetS in association with haplotype 2, which is marked by the polymorphism *Bcl*I, and a decreased prevalence of MetS in haplotype 5, which is marked by ER22/23EK. Several factors could contribute to the lack of associations in the present study. It could be speculated that haplotypes 2 and 5 lead to such subtle differences in glucocorticoid sensitivity that they do not result in an altered MetS prevalence. Both *Bcl*I and ER22/23EK have been associated with differences in negative feedback of glucocorticoids on the hypothalamus-pituitary-adrenal axis [9, 18]. A chronic slightly altered glucocorticoid sensitivity may therefore be partly compensated by altered cortisol levels, in which a relative hypersensitivity results in lower cortisol levels (in haplotype 2), and hyposensitivity results in increased cortisol (haplotype 5), thereby offsetting the change in glucocorticoid sensitivity. Another explanation for the lack of effect of haplotype 2 and 5 may be that we did not examine individuals at an age during which they are particularly at risk of the modulatory effects of these haplotypes on MetS presence. ER22/23EK presence has been associated with beneficial effects on the lipid profile in men above 53 years of age [18], while our present cohort mainly consists of adults of a younger age. Likewise, the *Bcl*I polymorphism was recently associated with increased insulin resistance and body weight, but this was in a population with a mean age of 65 with a high rate of type 2 diabetes mellitus [10].

In conclusion, we found that GR receptor haplotype 4, which is known to be associated with increased glucocorticoid sensitivity and increased transactivational activity of the GR, is associated with an increase in metabolic syndrome prevalence, specifically in young male adults and in individuals with low education status. These findings suggest that the association between glucocorticoid signaling and metabolic health is modulated by age, sex and socioeconomic status.

REFERENCES

1. Eckel, R.H., S.M. Grundy, and P.Z. Zimmet, *The metabolic syndrome.* Lancet, 2005. **365**(9468): p. 1415-28.
2. Mottillo, S., et al., *The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis.* J Am Coll Cardiol, 2010. **56**(14): p. 1113-32.
3. Grundy, S.M., *Metabolic syndrome pandemic.* Arterioscler Thromb Vasc Biol, 2008. **28**(4): p. 629-36.
4. Quax, R.A., et al., *Glucocorticoid sensitivity in health and disease.* Nature Reviews Endocrinology, 2013. **9**(11): p. 670-686.
5. Nieman, L.K., et al., *The diagnosis of Cushing's syndrome: an endocrine society clinical practice guideline.* The Journal of Clinical Endocrinology & Metabolism, 2008. **93**(5): p. 1526-1540.
6. Wester, V.L. and E.F. van Rossum, *Clinical applications of cortisol measurements in hair.* Eur J Endocrinol, 2015. **173**(4): p. M1-10.
7. Incollingo Rodriguez, A.C., et al., *Hypothalamic-pituitary-adrenal axis dysregulation and cortisol activity in obesity: A systematic review.* Psychoneuroendocrinology, 2015. **62**: p. 301-18.
8. Wester, V.L., S.W. Lamberts, and E.F. van Rossum, *Advances in the assessment of cortisol exposure and sensitivity.* Curr Opin Endocrinol Diabetes Obes, 2014. **21**(4): p. 306-11.
9. Van Rossum, E.F.C., et al., *Identification of the Bc/I polymorphism in the glucocorticoid receptor gene: Association with sensitivity to glucocorticoids in vivo and body mass index.* Clin Endocrinol (Oxf), 2003. **59**(5): p. 585-592.
10. Geelen, C.C., et al., *BclI glucocorticoid receptor polymorphism is associated with greater body fatness: the Hoorn and CODAM studies.* J Clin Endocrinol Metab, 2013. **98**(3): p. E595-599.
11. Ukkola, O., et al., *Interactions among the glucocorticoid receptor, lipoprotein lipase and adrenergic receptor genes and abdominal fat in the Quebec Family Study.* Int J Obes, 2001. **25**(9): p. 1332-1339.
12. Huizenga, N.A.T.M., et al., *A polymorphism in the glucocorticoid receptor gene may be associated with an increased sensitivity to glucocorticoids in vivo.* J Clin Endocrinol Metab, 1998. **83**(1): p. 144-151.
13. Russcher, H., et al., *Two polymorphisms in the glucocorticoid receptor gene directly affect glucocorticoid-regulated gene expression.* J Clin Endocrinol Metab, 2005. **90**(10): p. 5804-5810.
14. Charmandari, E., et al., *A novel point mutation in the amino terminal domain of the human glucocorticoid receptor (hGR) gene enhancing hGR-mediated gene expression.* The Journal of Clinical Endocrinology & Metabolism, 2008. **93**(12): p. 4963-4968.
15. Newton, R. and N.S. Holden, *Separating transrepression and transactivation: a distressing divorce for the glucocorticoid receptor?* Mol Pharmacol, 2007. **72**(4): p. 799-809.
16. Kuningas, M., et al., *Genetic variants in the glucocorticoid receptor gene (NR3C1) and cardiovascular disease risk. The Leiden 85-plus Study.* Biogerontology, 2006. **7**(4): p. 231-8.
17. Marti, A., et al., *Meta-analysis on the effect of the N363S polymorphism of the glucocorticoid receptor gene (GRL) on human obesity.* BMC Med Genet, 2006. **7**: p. 50.
18. Van Rossum, E.F.C., et al., *A polymorphism in the glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids in vivo, is associated with low insulin and cholesterol levels.* Diabetes, 2002. **51**(10): p. 3128-3134.
19. Van Rossum, E.F.C., et al., *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, 2004. **89**(8): p. 4004-4009.
20. van Rossum, E.F.C., et al., *Glucocorticoid receptor variant and risk of dementia and white, matter lesions.* Neurobiol Aging, 2008. **29**(5): p. 716-723.

21. Van Den Akker, E.L.T., et al., *Brief report: Glucocorticoid receptor polymorphism affects transrepression but not transactivation.* J Clin Endocrinol Metab, 2006. **91**(7): p. 2800-2803.
22. van den Akker, E.L.T., et al., *Glucocorticoid receptor gene and risk of cardiovascular disease.* Arch Intern Med, 2008. **168**(1): p. 33-39.
23. Koper, J.W., E.F. van Rossum, and E.L. van den Akker, *Glucocorticoid receptor polymorphisms and haplotypes and their expression in health and disease.* Steroids, 2014.
24. Stolk, R.P., et al., *Universal risk factors for multifactorial diseases: LifeLines: a three-generation population-based study.* Eur J Epidemiol, 2008. **23**(1): p. 67-74.
25. Alberti, K.G., et al., *Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity.* Circulation, 2009. **120**(16): p. 1640-5.
26. Alberti, K.G.M.M., P. Zimmet, and J. Shaw, *Metabolic syndrome—a new world-wide definition. A consensus statement from the international diabetes federation.* Diabetic medicine, 2006. **23**(5): p. 469-480.
27. Purcell, S., et al., *PLINK: a tool set for whole-genome association and population-based linkage analyses.* The American Journal of Human Genetics, 2007. **81**(3): p. 559-575.
28. Stephens, M., N.J. Smith, and P. Donnelly, *A new statistical method for haplotype reconstruction from population data.* Am J Hum Genet, 2001. **68**(4): p. 978-89.
29. Stephens, M. and P. Donnelly, *A comparison of bayesian methods for haplotype reconstruction from population genotype data.* Am J Hum Genet, 2003. **73**(5): p. 1162-9.
30. Park, Y.-W., et al., *The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994.* Archives of internal medicine, 2003. **163**(4): p. 427-436.
31. Rippe, R.C., et al., *Splitting hair for cortisol? Associations of socio-economic status, ethnicity, hair color, gender and other child characteristics with hair cortisol and cortisone.* Psychoneuroendocrinology, 2015. **66**: p. 56-64.
32. Klijs, B., et al., *Representativeness of the LifeLines Cohort Study.* PloS one, 2015. **10**(9): p. e0137203.