Glucocorticoid Receptor Gene and Risk of Cardiovascular Disease

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Background: Genetic variants in immunomodulating genes have been suggested to contribute to the risk of cardiovascular disease. Glucocorticoids are important regulators of inflammatory processes and the immune system. Our aim was to determine the contribution of genetic glucocorticoid receptor variants, with different cortisol sensitivities, to the risk of cardiovascular disease.

Methods: The study was conducted in a large (n=7983) population-based, prospective cohort of the Rotterdam Study. The mean duration of follow-up was 8.9 years. Measures of cardiovascular disease were incident myocardial infarction, coronary heart disease, high-sensitivity C-reactive protein level, interleukin 6 level, and carotis intima-media thickness.

Results: Persons homozygous for haplotype 3, which is a common variant of the glucocorticoid receptor gene, had a more than 2-fold increased risk of myocardial infarction (hazard ratio, 2.1; 95% confidence interval, 1.13-4.07) and an almost 3-fold increased risk of coronary heart disease (hazard ratio, 2.6; 95% confidence interval, 1.40-4.81) compared with nonhomozygous persons. In addition, their C-reactive protein and interleukin 6 levels were higher, and carotis intima-media thickness was greater. No associations were found for the other haplotypes.

Conclusions: The glucocorticoid receptor gene haplotype 3 is a common genetic variant and is related to a more active proinflammatory system. This haplotype is associated with the risk of cardiovascular disease and its parameters. These results should be regarded as hypothesis generating until they have been replicated in other studies. Our findings suggest that genetically determined cortisol sensitivity is involved in the pathogenesis of cardiovascular disease and might identify a subgroup at risk.


GENETIC SUSCEPTIBILITY

for cardiovascular disease has been found for diverse systems, such as the hemostatic system, the metabolism of lipids and glucose, and the action of sex hormones.1,2 Recently, interest in immunogenetic susceptibility for atherosclerosis has been increasing because inflammation and immune response have been found to contribute to atherogenesis.3 Several proteins of the innate immune response were found to predict cardiovascular risk, the most important being C-reactive protein (CRP).3,4 Glucocorticoids are important regulators of the immune system, inflammatory processes, and many other processes involved in fat and glucose metabolism and the cardiovascular system. Several studies showed that high levels of glucocorticoids result in unfavorable cardiovascular risk factors, such as visceral obesity, steroid-induced diabetes, and hypercholesterolemia.5 Glucocorticoids exert their effect through the glucocorticoid receptor, which is expressed in most cells of the human body and regulates the expression of multiple genes.5 The sensitivity to glucocorticoids varies considerably between individuals.6 Polymorphisms in the glucocorticoid receptor gene are thought to play a role in this.7 Four glucocorticoid receptor gene variants have been associated with a change in cortisol sensitivity: BclI, N363S, ER22/23EK, and GR-9β.7 Three of these polymorphisms (ER22/23EK, N363S, and BclI) have been studied in a population of 552 elderly persons for their risk on cardiovascular disease and no association was found.8 However, literature reports are conflicting.9 Replication
or validation of findings from genetic association studies is needed.

In recent articles we reported on the aforementioned 4 glucocorticoid receptor gene variants and their haplotypes\textsuperscript{10,11} (Figure 1). Our finding of decreased immunosuppression in haplotype 3 (GR-9β polymorphism) carriers\textsuperscript{10,11} led to the a priori hypothesis that these persons might have elevated inflammatory parameters and an increased risk of cardiovascular disease through a lifelong exposure to diminished cortisol suppression of the pro-inflammatory system. Our aim was to examine the association of glucocorticoid receptor variants, which have been associated with changes in cortisol sensitivity, with different measures of atherosclerosis, such as high-sensitivity CRP (hs-CRP) levels, interleukin 6 (IL-6) levels, carotis intima-media thickness, and risk of myocardial infarction (MI) and coronary heart disease (CHD). This association was examined in a large population-based follow-up study.

\section*{METHODS}

\textbf{STUDY POPULATION}

This study was conducted as part of the Rotterdam Study, a population-based, prospective cohort study on determinants of disease and disability in 7983 persons 55 years and older and living in Rotterdam, the Netherlands.\textsuperscript{12} Our study population included men and women 55 years and older, who were white, were able to visit the research center, and completed all parts

\begin{figure}
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Schematic overview of the glucocorticoid receptor (GR) gene polymorphisms and haplotypes. Haplotypes are numbered in order of decreasing frequency. The nucleic acid changes are indicated. TAD indicates transactivating domain; DBD, DNA-binding domain; LBD, ligand-binding domain; G, guanidine; A, adenosine; and C, cytidine.}
\end{figure}
of the baseline examination and provided a blood sample. The Medical Ethics Committee of the Erasmus Medical Center approved the study. Informed consent and permission to retrieve information from treating physicians was obtained from all participants.

MEASUREMENTS

A trained interviewer visited all subjects at home and collected information on current health status, medical history, drug use, and smoking using a computerized questionnaire. Cardiovascular risk factors were obtained by interview and physical examination at baseline as described previously. A history of MI was considered present in the case of a self-report of MI, confirmed by an electrocardiogram (ECG) or additional clinical information, or in the case of an ECG characteristic of prior MI. Interview information included smoking habits, age at menopause, and medication use, including hormone therapy. Smoking was categorized as current, past, or never smoker. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Hypertension was defined as a systolic blood pressure of 160 mm Hg or greater, and/or the use of antihypertensive medication, encompassing grade 2 and grade 3 hypertension according to the World Health Organization (WHO) criteria. After an overnight fast, blood samples were obtained. Diabetes mellitus was considered present with current use of antidiabetic medication or a nonfasting or postload glucose level of 190 mg/dL (to convert to millimoles per liter, multiply by 0.0555) or higher according to the WHO criteria. As a measure of atherosclerosis, intima-media thickness was measured by ultrasonography as described previously.

Serum, stored at −20°C, was used for the measurement of hs-CRP by a nephelometric method (Dade Behring, Marburg, Germany) as described elsewhere. The detection limit of the assay was 0.02 mg/L (to convert to nanomoles per liter, multiply by 0.0555) or higher according to the WHO criteria. As a measure of atherosclerosis, intima-media thickness was measured by ultrasonography as described previously.

We used the genotype data for each of the 4 polymorphisms to infer the haplotypes present in the population using the program PHASE, which implements a Bayesian statistical method for reconstructing haplotypes from population genotype data. Of the 4 polymorphisms, 3 were found to be mutually exclusive; only the codon 23 A allele was always present in combination with the GR-9β G allele. The alleles were defined as haplotypes such as “GG-A-C-A” (haplotype 1) representing a guanidine (G) nucleotide at the 198 and 200 G → A polymorphic site (rs 6189 and rs 6190, respectively); an adenosine (A) nucleotide at the A220 A → G polymorphic site (rs 6193); a cytidine (C) nucleotide at the C → G Bcl polymorphic site; and an adenosine (A) at the A → G GR-9β polymorphic site (rs 6198) (Figure 1). For each haplotype, 3 genotype combinations were distinguished as carrying 0, 1, or 2 copies of the haplotype allele. Haplotype 1 carries the major alleles of the polymorphisms; therefore, the reference allele is defined as carrying 2 copies of haplotype 1.

FOLLOW-UP PROCEDURES

Follow-up data on MI and CHD were collected from baseline (1990-1993) until January 1, 2003. Cardiovascular disease morbidity and mortality were reported by general practitioners in the Rotterdam area by means of a computerized system. In the case of recurrent MI or CHD during the follow-up period, the first event was used in the analysis. All reported events were verified by research physicians who collected information from the patients' medical records. This information and information on revascularization procedures were also identified by consulting hospital discharge letters from the medical specialist. All events were coded independently by 2 research physicians according to the International Statistical Classification of Diseases, 10th Revision (ICD-10). Finally, a medical expert in cardiovascular disease reviewed all coded events for final classification. All available information, including ECG, cardiac enzymes, and the clinical judgment of the treating specialist, was used to code the events. In the present study, we used the following end points: incident MI (ICD-10 code I21) and CHD. Coronary heart disease was defined as MI (ICD-10 code I21), percutaneous transluminal coronary angioplasty (ICD-10 code Z95.5), coronary artery bypass graft (ICD-10 code Z95.1), and death from CHD (ICD-10 codes I20-125).

POPULATION FOR ANALYSIS

A total of 7129 participants visited the research center. DNA was available for 6571 subjects. Reasons for nonavailability of DNA were failure of venipuncture, failure of DNA isolation in the laboratory or failure of allelic discrimination by TaqMan analysis (approximately 5% per polymorphism), or incomplete haplotypes (7%). For only 7 persons, follow-up data on CHD were missing.

Genotyping of all 4 polymorphisms was successful in 6081 subjects. For 6074 of these subjects, follow-up data on CHD were complete. Persons with a history of MI at baseline were excluded (n = 1196). In total, 4878 subjects were available for analysis of the association between haplotype and incident CHD. For hs-CRP and IL-6 analysis, subjects with possible acute in-
flammation as indicated by an hs-CRP level greater than >1 mg/L or an IL-6 level greater than 10 pg/mL were excluded. In the study population, 5.0% of the hs-CRP levels were greater than 1 mg/L, and 2.9% of the IL-6 levels were greater than 10 pg/mL.

STATISTICAL ANALYSIS

Differences in baseline characteristics between carriers of 0, 1, and 2 copies of haplotype 1 to 5 or between men and women were examined by 1-way analysis of variance (continuous variables) and Pearson χ² (dichotomous variables). Because of the low numbers of homozygous subjects for haplotype 4 (n=6) and 5 (n=8), these haplotypes were analyzed as carriers (1 or 2 copies) and noncarriers (0 copies). Results are reported as means [SDs]. We used logarithmically transformed values of CRP and IL-6 to normalize the distribution of these variables. The association between the glucocorticoid receptor haplotypes and CHD events was evaluated by age-adjusted Cox proportional hazards model. Hazard ratios were computed as estimates of relative risk. To account for possible confounding, we excluded all subjects with previous MI at baseline (model 1) and computed relative risks in a multivariate model containing the following variables: age and sex (model 1); model 1 variables plus BMI, systolic blood pressure, smoking, diabetes mellitus, total and high-density lipoprotein cholesterol levels (model 2); and model 2 variables plus hs-CRP (model 3).

Baseline characteristics of the study population are described in Table 1. The median age was 68.5 years. Frequencies of the haplotype alleles are presented in Figure 1. Owing to their high linkage disequilibrium, 3 of the 4 described polymorphisms are mutually exclusive. The distribution of genotypes for all glucocorticoid receptor gene polymorphisms was in Hardy-Weinberg equilibrium (P > .05). Haplotypes 1, 2, and 3 were most frequent with allele frequencies of 41.9%, 36.5%, and 14.5%, respectively. Haplotypes 4 and 5 had allele frequencies of 3.7% and 3.4%, respectively. Comparison of means of baseline characteristics between carriers of 0, 1, and 2 copies of haplotype 1 to 5 revealed no significant differences for the covariates.

The mean (SD) duration of follow-up was 8.9 (3.2) years (range, 0.05-13.5 years). During the follow-up period, an incident MI occurred in 220 participants (4.5%), and 493 of the participants (10.1%) had a CHD event. Myocardial infarction and CHD events were more frequent in men (MI, n=135 [7.2%]; and CHD, n=257 [13.7%]) compared with women (MI, n=85 [2.8%]; and CHD, n=236 [7.8%]). No association with MI or CHD was observed for glucocorticoid receptor haplotypes 1, 2, 4, or 5 (Table 2). Persons homozygous for haplotype 3 had an increased risk of MI (P = .04) as well as CHD (P = .03) compared with nonhomozygous individuals. Adjustments for age and sex (model 1) and subsequently for BMI, systolic blood pressure, smoking, diabetes mellitus, and total and high-density lipoprotein cholesterol levels (model 2) strengthened these associations.
Figure 2 presents the cumulative risk of incident CHD during the follow-up period for persons carrying 0, 1, or 2 copies of haplotype 3, adjusted for cardiovascular risk factors. Additional correction for hs-CRP level in model 3 slightly lowered the risk of MI or CHD (hazard ratios of 2.10 for MI and 2.73 for CHD). The risk of CHD remained significantly elevated after the exclusion of incident MI (hazard ratio, 3.3; 95% CI, 1.42-7.68 [P = .005]).

In sex-specific subgroup analyses, the numbers of events were too small to justify statistical interpretation. High-sensitivity CRP level, IL-6 level, and intima-media thickness were significantly higher in homozygous carriers of haplotype 3 (_table 3). No association was found between haplotype 3 and frequency of diabetes mellitus (data not shown). No association was observed between hs-CRP level, IL-6 level, or intima-media thickness and haplotypes 1, 2, 4, or 5 (data not shown).

COMMENT

In this large population-based follow-up study, we found a common glucocorticoid receptor haplotype to be associated with an increased risk of MI and CHD. Persons homozygous for haplotype 3 had a 2.2-fold increased risk of MI and a 2.8-fold increased risk of CHD compared with...
The glucocorticoid receptor gene, which is transcribed in the
nose and might be more susceptible to rheumatoid arthritis, which suggests that the GR-9β polymorphism can lead to a more active immune system. Myocardial infarction, CHD, and rheumatoid arthritis all have an inflammatory pathogenesis. We hypothesize that glucocorticoid receptor haplotype 3 leads to a higher risk of MI and CHD through a lifelong exposure to diminished cortisol suppression of the proinflammatory system.

There are multiple pathways through which altered glucocorticoid receptor sensitivity could influence these inflammatory processes. Glucocorticoids inhibit the immune response and the proinflammatory response by, for example, suppression of the synthesis of cytokines and inflammatory mediators such as nuclear factor κB, which is a key regulator in the process of atherosclerosis. Thus, they act on a variety of immune cells to control inflammation. Moreover, glucocorticoids influence vasodilatation through decreased nitric oxide release, vascular permeability, and migration of leukocytes across the endothelium, which are all important factors in inflammation and atherogenesis. Second, the glucocorticoid receptor can regulate the expression of toll-like receptors, which are known to activate signaling pathways to inflammation and are associated with CRP level and CHD.

Our finding of a relationship of a common glucocorticoid receptor genetic variant with the risk of MI and CHD and other parameters of atherosclerosis might have important implications for our understanding of the inflammatory pathogenesis of CHD. Possibly, glucocorticoid receptor genotype analysis can identify a subgroup that may in particular benefit from anti-inflammatory treatment.

Table 3. High-Sensitivity C-Reactive Protein (hs-CRP) Levels, IL-6 Levels, and Carotis IMT for Glucocorticoid Receptor Haplotype 3

<table>
<thead>
<tr>
<th>Copies</th>
<th>No. of Persons</th>
<th>Mean (SEM)</th>
<th>P Value</th>
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</thead>
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<tr>
<td></td>
<td>hs-CRP</td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>4020</td>
<td>0.24 [0.03]</td>
<td>mg/L</td>
</tr>
<tr>
<td>1</td>
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<td>mg/L</td>
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<tr>
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<td>121</td>
<td>0.28 [0.02]</td>
<td>mg/L</td>
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<tr>
<td></td>
<td>IL-6</td>
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<td>434</td>
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<td>pg/mL</td>
</tr>
<tr>
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<td>pg/mL</td>
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<tr>
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<td>2</td>
<td>91</td>
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<td>mm</td>
</tr>
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</table>

Abbreviations: IL-6, interleukin 6; IMT, intima-media thickness.
SI conversion factor: To convert hs-CRP to nanomoles per liter, multiply by 9.524.

a P values were derived from analysis of variance, natural log transformed, Bonferroni corrected for multiple testing, and corrected for age, body mass index, and smoking.

b P value by nonparametric test (Kruskal-Wallis).

Nonhomozygous individuals. The increase in risk prediction achieved with haplotype 3 was similar to that reported for other risk markers such as cholesterol or hs-CRP level. In addition, homozygous carriers of haplotype 3 showed significantly higher levels of hs-CRP and IL-6 and greater carotis intima-media thickness (Table 3). No associations were found for the other haplotypes.

One of the major limitations of genetic association studies is lack of reproducibility. Although the numbers of events for MI and CHD in our study are limited, the results are further supported by the significant association that was found for hs-CRP and IL-6 levels as important inflammatory parameters and by intima-media thickness as a measure of atherosclerosis (Table 3). These results were in line with our prior hypothesis based on previous studies. The GR-9β polymorphism of haplotype 3 is located in the 3’ untranslated region of the glucocorticoid receptor gene, which is transcribed in the GRβ splice variant thought to have a dominant negative effect on GRα. In vitro data show that this polymorphism leads to increased GRβ expression by a more stable GRβ messenger RNA transcript and thereby to relative glucocorticoid resistance. Indeed, persons carrying haplotype 3 seem to have a decreased glucocorticoid transrepression with a normal transactivation. The influence of glucocorticoids on the immune system is regulated through transrepression. Clinical data show that persons homozygous for haplotype 3 had a reduced risk of bacterial colonization with Staphylococcus aureus in the nose and might be more susceptible to rheumatoid arthritis, which suggests that the GR-9β polymorphism can lead to a more active immune system. Myocardial infarction, CHD, and rheumatoid arthritis all have an inflammatory pathogenesis. We hypothesize that glucocorticoid receptor haplotype 3 leads to a higher risk of MI and CHD through a lifelong exposure to diminished cortisol suppression of the proinflammatory system.

In conclusion, the glucocorticoid receptor gene haplotype 3, which is related to a diminished suppressive effect of cortisol on the proinflammatory system, is associated with the risk of MI and CHD. This is further supported by higher levels of hs-CRP and IL-6, which are important markers for cardiovascular disease and higher carotis intima-media thickness as a measure of atherosclerosis. These findings may provide new insight in our understanding of the inflammatory pathogenesis of cardiovascular disease and might identify a subgroup at risk.
REFERENCES

7. van Rossum EF, Lamberts SW. Polymorphisms in the glucocorticoid receptor gene and their associations with metabolic parameters and body composition. Recent Prog Horm Res. 2004;59:333-357.