Gene-load score of the renin–angiotensin–aldosterone system is associated with coronary heart disease in familial hypercholesterolaemia

Jeroen B. van der Net1,2, Jeroen van Etten1,2, Mojgan Yazdanpanah1, Geesje M. Dallinga-Thie3, John J.P. Kastelein3, Joep C. Defesche3, Richard P. Koopmans4, Ewout W. Steyerberg2, and Eric J.G. Sijbrands1*

1Department of Internal Medicine—D435, Erasmus MC, University Medical Center, PO Box 2040, 3000 CA Rotterdam, The Netherlands; 2Department of Public Health, Erasmus MC, University Medical Center, Rotterdam, The Netherlands; 3Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands; and 4Department of Internal Medicine, Academic Hospital Maastricht, Maastricht, The Netherlands

Received 3 October 2007; revised 18 March 2008; accepted 20 March 2008; online publish-ahead-of-print 14 April 2008

Aims
Familial hypercholesterolaemia (FH) is characterized by premature coronary heart disease (CHD). However, the incidence of CHD varies considerably among FH patients. Genetic variation in the renin–angiotensin–aldosterone system (RAAS) and the adrenalin/noradrenalin system may be of importance in determining the CHD risk in FH, because of their involvement in CHD. We investigated the association between CHD risk and combined genetic variation in the RAAS and adrenalin/noradrenalin system.

Methods and results
In 2190 FH patients, we genotyped six RAAS polymorphisms and five adrenalin/noradrenalin polymorphisms. For each patient, we calculated two gene-load scores by counting the number of risk genotypes within each pathway. Four of the six RAAS polymorphisms and none of the polymorphisms in the adrenalin/noradrenalin system were significantly associated with CHD (P < 0.05). The RAAS gene-load score was significantly associated with CHD (Plinear trend < 0.001): in patients with a gene-load score of 5 or 6, the CHD risk was 2.3 times as high as in patients with a score of 0 or 1. The gene-load score of the adrenalin/noradrenalin system was not associated with CHD.

Conclusion
Genetic variation in the RAAS contributes gene-dose dependently to CHD risk in patients with FH, whereas genetic variation in the adrenalin/noradrenalin system is not associated with CHD.

Keywords
Coronary heart disease • Familial hypercholesterolaemia • Genetics • Polymorphism • Renin–angiotensin–aldosterone system • Adrenalin/noradrenalin system

Introduction
Familial hypercholesterolaemia (FH) is an autosomal dominant disorder caused by mutations in the low-density lipoprotein (LDL) receptor gene.1 Clinically, the disease is characterized by severely elevated LDL cholesterol levels, tendon xanthomas, and premature coronary heart disease (CHD).2 Although FH is a monogenic disorder, there is considerable variation in the onset of cardiovascular disease and all-cause mortality,3 even among carriers of an identical LDL receptor mutation.4,5 Classical risk factors have been shown to influence the burden of FH. In addition, modifier genes may be of importance.6,7

The renin–angiotensin–aldosterone system (RAAS) and adrenalin/noradrenalin system are involved in the development of atherosclerosis and CHD.8–10 Therefore, the genes of these pathways are important candidate genes for CHD. However, studies conducted so far showed conflicting results concerning the associations between single-nucleotide polymorphisms in genes involved in these pathways and CHD.11,12 These discrepancies could be explained by the fact that the RAAS and adrenalin/noradrenalin system are regulated by a large number of interacting genes, resulting in highly redundant regulation systems. Most likely, genetic variation is only relevant when compensation fails. Therefore, it is appropriate to consider the combined effects of multiple genes.
when studying the effect of complex pathways on complex phenotypes such as CHD.13

We hypothesized that combination of multiple genetic variants of the RAAS and adrenalin/noradrenalin pathways is associated with CHD risk in FH patients. We investigated the combined effect of six RAAS polymorphisms and the combined effect of five adrenalin/noradrenalin polymorphisms in relation to CHD risk in a large cohort of FH patients.

Methods

Study design, population, and data collection

We performed a multicentre cohort study among heterozygous FH patients who were recruited from 27 lipid clinics in the Netherlands between 1989 and 2002. More detailed information on the study design and the study population was published previously.14,15 In brief, the DNA of suspected FH individuals from lipid clinics in the Netherlands is routinely submitted to a central laboratory for LDL receptor mutation analysis. We randomly selected 2400 FH patients (99% Caucasian) who fulfilled the internationally established FH diagnostic criteria,14 and were apparently unrelated, which was confirmed by making pedigrees up to third-degree relatives. The DNA of 2190 patients was available for the present analysis, because the DNA of the remaining patients was insufficient in amount and/or quality for use in genetic studies. Medical records were used to acquire information about age, sex, smoking, body mass index (BMI), presence of diabetes mellitus, and plasma lipid parameters.14,15 Blood pressure measurements were based on single-office visits. We used the diagnosis hypertension (documented diagnosis with antihypertensive medication or systolic blood pressure $\geq 140$ mmHg and/or diastolic blood pressure $\geq 90$ mmHg at three consecutive office visits) in our analyses to ensure that deliberate clinical evaluation had taken place. The median follow-up time at the lipid clinic was 3.1 years. All patients gave informed consent and the ethics institutional review board of each participating hospital approved the protocol.

Coronary heart disease definition

The endpoint was proven CHD, which was defined by the presence of at least one of the following: (i) myocardial infarction, proved by at least two of the following: (a) classical symptoms ($\geq 15$ min), (b) specific ECG abnormalities, and/or (c) elevated cardiac enzymes ($\geq 2 \times$ upper limit of normal); (ii) percutaneous coronary intervention or other invasive procedures; (iii) coronary artery bypass grafting; (iv) angina pectoris, diagnosed as classical symptoms in combination with at least one unequivocal result of one of the following: (a) exercise test, (b) nuclear scintigram, (c) dobutamine stress ultrasound, or (d) $>70\%$ stenosis on a coronary angiogram.

Genetic analyses

A total of six polymorphisms in genes involved in RAAS and five polymorphisms in genes involved in the adrenalin/noradrenalin system were selected as candidate genes. The $825C\rightarrow T$ polymorphism in the $G$ protein $\beta 3$ subunit gene was involved in both the RAAS and the adrenalin/noradrenalin system. All selected polymorphisms have been associated with CHD, myocardial infarction, blood pressure, and/or hypertension (see Supplementary material online 1 for relative risk estimates for CHD in non-FH populations). Seven of the 10 polymorphisms were analysed with a multilocus genotyping assay based on a probe mismatch hybridization method that was published previously: 4072T$\rightarrow$C (Met235Thr, rs699) in the angiotensinogen (AGT) gene,16 insertion/deletion polymorphism (rs1799752) in intron 16 in the angiotensin-converting enzyme (ACE) gene,1,17 1166A$\rightarrow$C (rs5186) in the angiotensin II type 1 receptor (AGTR1) gene,16,19 614G$\rightarrow$T (Gly460Tyr, rs4961) in the $\alpha$-adducin (ADD1) gene,20 46G$\rightarrow$A (Arg16Gly, rs1042713) and 79C$\rightarrow$G (Gln27Glu, rs1042714) polymorphisms in the adrenergic B2 receptor (ADRB2) gene,12,14,21 and 825C$\rightarrow$T (rs5443) in the $\beta$ protein $\beta 3$ subunit (GNB3) gene.21 We genotyped the $344C\rightarrow T$ polymorphism (rs1799998) in the adrenalin sytem (CYP1B1) gene,23 1039C$\rightarrow$T (Arg347Cys, rs1048101) in the adrenergic receptor 1a (ADRA1A) gene,24 and 145A$\rightarrow$G (Ser49Gly, rs1801252) in the $\beta$1-adrenergic receptor (ADRB1) gene.25 The latter genotypes were determined using the fluorescence-based assay-by-design allele discrimination method using TaqMan Universal PCR master mix (Applied Biosystems, Foster City, USA) and a Taqman ABI Prism 7900 Sequence Detection System (Applied Biosystems). Primer and probe sequences of the genotyped polymorphisms are presented in Supplementary material online 2. Reaction components and amplification parameters were based on the manufacturer’s instructions using an annealing temperature of 60°C. Results were scored blinded to CHD status. The mean success rate of the genotyping was 93%.

Gene-load score

The combined effect of genes can be examined by calculating a gene-load score, which measures the number of risk genotypes within a pathway for an individual. In this way, the gene-load score gives a reflection of an individuals’ ‘genetic burden’ and represents the combined effect of genetic variation in multiple genes within a pathway.13 The effect of the gene-load scores of the two pathways on CHD risk was our primary analysis to test our hypothesis. For each patient, we computed the gene-load scores of the RAAS and adrenalin/noradrenalin system. The mode of inheritance of each polymorphism was chosen on the basis of the literature. The dominant genetic model was chosen for the polymorphisms in the AGTR1, ADD1, ADRA1A, and ADRB2 genes,11,12,23,25 whereas the recessive model was chosen for the polymorphisms in the AGT, ACE, CYP1B1, and ADRB1 genes.11,12,23,25 For the polymorphism in the GNB3 gene, the literature was inconclusive.22,26 Therefore, we chose the recessive mode of inheritance on the basis of the genotypic test (2 df) and Armitage trend test (1 df).

Since we genotyped six polymorphisms in the RAAS, the overall RAAS gene-load score could vary between 0 and 6. The highest RAAS gene-load score category 6 had a low number of patients ($n=3$) and was therefore combined with category 5. Similarly, category 0 showed a low number ($n=14$) and was combined with category 1. The same procedure was used for the gene-load score of the adrenalin/noradrenalin system. A reversed coding was applied for the GNB3 polymorphism, because it associated with lower CHD risk: the risk genotypes (CC + CT) were coded as 1 and the TT genotype was coded as 0. Both gene-load scores were analysed, with the lowest gene-load score category as reference category. We assigned the same score to each risk genotype, because it was shown that this yields the same predictive accuracy as a score on the basis of the individual effects of the risk genotypes on CHD.7 The selected genes were located on different chromosomes. Therefore, the calculation of the score was not complicated by linkage disequilibrium and represents a multilocus effect.

Statistical analyses

Statistical analyses were performed with the SPSS for Windows 12.0.1 statistics program. For differences between groups, we used the
We assessed the association of each polymorphism and gene-load score with CHD by Cox proportional hazards regression. For associations between the gene-load scores and CHD, we tested a linear trend by coding these scores as ordinal variables. We used the Cox proportional hazards model, because the age at event was known as well as multivariable with adjustment for age at last visit to the lipid clinic, sex, and smoking status.

Results

Patient characteristics

The clinical characteristics of the 2190 patients are presented in Table 1 together with the cumulative risk of CHD till the age of 40, 50, and 60 years. Over a total of 108 925 person-years, 618 (28%) patients had at least one CHD event. The mean age of onset of the first CHD event was 48.8 years. The following variables were significantly associated with a higher cumulative CHD risk: male gender, smoking, plasma total, HDL and LDL cholesterol levels below the mean, and plasma triglyceride levels above the mean (Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics and outcome of 2190 patients with familial hypercholesterolaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical characteristic</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex*</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>Smoking*</td>
<td>Never</td>
</tr>
<tr>
<td></td>
<td>Ever</td>
</tr>
<tr>
<td>Hypertension</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>BMI</td>
<td>≤ 25</td>
</tr>
<tr>
<td></td>
<td>25 &lt; BMI ≤ 30</td>
</tr>
<tr>
<td></td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Total cholesterol**</td>
<td>≤ 9.20 mmol/L</td>
</tr>
<tr>
<td></td>
<td>&gt; 9.20 mmol/L</td>
</tr>
<tr>
<td>LDL cholesterol**</td>
<td>≤ 6.99 mmol/L</td>
</tr>
<tr>
<td></td>
<td>&gt; 6.99 mmol/L</td>
</tr>
<tr>
<td>HDL cholesterol*</td>
<td>≥ 1.16 mmol/L</td>
</tr>
<tr>
<td></td>
<td>≤ 1.16 mmol/L</td>
</tr>
<tr>
<td>Triglycerides**</td>
<td>≤ 1.57 mmol/L</td>
</tr>
<tr>
<td></td>
<td>&gt; 1.57 mmol/L</td>
</tr>
<tr>
<td>Total</td>
<td>2190</td>
</tr>
</tbody>
</table>

For total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides, we used the median to split the total population into two subpopulations. CHD, coronary heart disease; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

*P < 0.001, **P < 0.05.
Polymorphisms and coronary heart disease

The genotype frequencies of the 10 polymorphisms are shown in Supplementary material online 3. All polymorphisms were in Hardy–Weinberg equilibrium, except for the ADD1 614G→T polymorphism (P = 0.01). The AGT, AGTR1, CYP11B2, and ADD1 polymorphisms were significantly associated with CHD in the primary model (Table 2). Adjustment for hypertension did not change the results, whereas after additional adjustment for diabetes mellitus, BMI, plasma HDL cholesterol, and plasma triglycerides, only the AGT, ACE, and ADD1 polymorphisms were associated with CHD (Table 2). None of the polymorphisms in the adrenalin/noradrenalin system was associated with CHD (Table 2). The associations between the polymorphisms and CHD in different inheritance models are presented in Supplementary material online 4.

We further investigated whether the associations between the polymorphisms involved in the two pathways and CHD were dependent on hypertension. No associations between the polymorphisms and hypertension were found (Supplementary material online 5).

Table 2 Association between renin–angiotensin–aldosterone system and adrenalin/noradrenalin polymorphisms and coronary heart disease

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Model 1 HR (95% CI)</th>
<th>P-value</th>
<th>Model 2 HR (95% CI)</th>
<th>P-value</th>
<th>Model 3 HR (95% CI)</th>
<th>P-value</th>
<th>PAR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGT 4072T→C</td>
<td>1.28 (1.02–1.61)</td>
<td>0.04</td>
<td>1.28 (1.02–1.62)</td>
<td>0.03</td>
<td>1.26 (1.01–1.58)</td>
<td>0.04</td>
<td>3.8</td>
</tr>
<tr>
<td>ACE I/D intron 16</td>
<td>1.18 (0.97–1.44)</td>
<td>0.1</td>
<td>1.19 (0.97–1.46)</td>
<td>0.09</td>
<td>1.23 (1.01–1.49)</td>
<td>0.04</td>
<td>6.0</td>
</tr>
<tr>
<td>AGTR1 1166A→C</td>
<td>1.21 (1.00–1.45)</td>
<td>0.048</td>
<td>1.22 (1.01–1.46)</td>
<td>0.04</td>
<td>1.12 (0.94–1.34)</td>
<td>0.2</td>
<td>NA</td>
</tr>
<tr>
<td>CYP11B2–344C→T</td>
<td>1.22 (1.01–1.48)</td>
<td>0.04</td>
<td>1.22 (1.00–1.47)</td>
<td>0.047</td>
<td>1.17 (0.97–1.41)</td>
<td>0.09</td>
<td>NA</td>
</tr>
<tr>
<td>ADD1 614G→T</td>
<td>1.22 (1.01–1.47)</td>
<td>0.04</td>
<td>1.22 (1.02–1.48)</td>
<td>0.03</td>
<td>1.25 (1.05–1.50)</td>
<td>0.01</td>
<td>8.5</td>
</tr>
<tr>
<td>GNB3 825C→T</td>
<td>0.72 (0.52–1.01)</td>
<td>0.06</td>
<td>0.73 (0.52–1.01)</td>
<td>0.06</td>
<td>0.78 (0.57–1.06)</td>
<td>0.1</td>
<td>NA</td>
</tr>
<tr>
<td>Adrenalin/noradrenalin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADRA1A 1039C→T</td>
<td>0.97 (0.77–1.23)</td>
<td>0.8</td>
<td>0.99 (0.78–1.25)</td>
<td>0.9</td>
<td>0.95 (0.76–1.18)</td>
<td>0.6</td>
<td>NA</td>
</tr>
<tr>
<td>ADRB1 145A→G</td>
<td>1.03 (0.51–2.08)</td>
<td>0.9</td>
<td>1.01 (0.50–2.04)</td>
<td>1.0</td>
<td>0.98 (0.48–1.98)</td>
<td>1.0</td>
<td>NA</td>
</tr>
<tr>
<td>ADRB2 46G→A</td>
<td>1.17 (0.97–1.41)</td>
<td>0.1</td>
<td>1.17 (0.97–1.42)</td>
<td>0.1</td>
<td>1.16 (0.96–1.39)</td>
<td>0.1</td>
<td>NA</td>
</tr>
<tr>
<td>ADRB2 79C→G</td>
<td>1.00 (0.82–1.22)</td>
<td>1.0</td>
<td>1.00 (0.82–1.22)</td>
<td>1.0</td>
<td>1.04 (0.86–1.26)</td>
<td>0.7</td>
<td>NA</td>
</tr>
<tr>
<td>GNB3 825C→T</td>
<td>0.72 (0.51–1.01)</td>
<td>0.053</td>
<td>0.73 (0.52–1.01)</td>
<td>0.06</td>
<td>0.78 (0.57–1.06)</td>
<td>0.1</td>
<td>NA</td>
</tr>
</tbody>
</table>

Model 1: Cox proportional hazards model adjusted for sex, year of birth, and smoking; model 2: additional adjustment for hypertension; model 3: additional adjustment for hypertension, diabetes mellitus, BMI, plasma HDL cholesterol, and plasma triglycerides; HR, hazard ratio; 95% CI, 95% confidence interval; PAR, population attributable risk, based on model 3. NA, not applicable, because polymorphism is not significantly associated with CHD in model 3.

Gene-load scores and coronary heart disease

The RAAS gene-load score was gene-dose dependently associated with CHD (Plinear trend < 0.001, Figure 1). CHD risk in patients with a gene-load score of 5 or 6 was 2.3 times as high as in patients with a gene-load score of 0 or 1 (95% CI 1.39–3.86, P = 0.001). The association between the RAAS gene-load score and CHD was not influenced by additional adjustment for hypertension, diabetes mellitus, BMI, plasma HDL cholesterol, and plasma triglycerides (data not shown). The PAR of the RAAS gene-load score was 26%.

The gene-load score of the adrenalin/noradrenalin system was not significantly associated with CHD (Plinear trend = 0.6, Figure 2). Additional adjustment for hypertension, diabetes mellitus, BMI, plasma HDL cholesterol, and plasma triglycerides did not change this result (Plinear trend = 0.3).

The gene-load scores were not associated with hypertension (Plinear trend = 0.3 for RAAS and Plinear trend = 0.6 for adrenalin/noradrenalin system, adjusted for age at last visit to the lipid clinic, sex, and smoking).

Discussion

This study demonstrates that variation in RAAS genes is gene-dose dependently associated with CHD in patients with FH. The RAAS gene-load score is a model for multilocus effects within a pathway related to CHD. The presence of at least five risk genotypes in the RAAS resulted in a CHD risk that was more than twice as high as that of one risk genotype or fewer. This study indicates that despite the fact that the individual polymorphisms revealed small effects, the combinations of polymorphisms may more clearly predict susceptibility to CHD.
Two previous studies investigated the association between genetic variation in the RAAS and CHD in FH populations and found significant associations for the ACE polymorphism and the AGTR1 polymorphism, which is in line with the present study. These previous studies, however, did not study the combined influence of these polymorphisms on CHD. In contrast, several studies in non-FH populations did investigate interactions between polymorphisms within the RAAS and their combined influence on cardiovascular disease outcomes. To our knowledge, the present study is the first to show that the multilocus effect of six genes involved in the RAAS substantially strengthens the association with CHD. Normally, feedback mechanisms within the RAAS counteract changes in individual components. For instance, a rise in angiotensinogen will be compensated by a decrease in renin, and, consequently, plasma and tissue angiotensin levels are unaltered under such conditions. However, in the presence of multiple risk genotypes, it will become increasingly difficult to normalize the degree of RAAS activity via feedback mechanisms. This most likely explains the current results. Furthermore, the association between the RAAS gene-load score and CHD occurred independently of hypertension. A hypertension-independent association between genetic variation in the RAAS and CHD has already been described in a population-based study, and this finding is therefore not necessarily specific for hypercholesterolaemia. Taken together, our observations suggest that the negative consequences of increased RAAS activity predominantly manifest themselves at the tissue level, in agreement with the concept of tissue angiotensin production. Such tissue effects involve multiple mechanisms that are all associated with atherosclerosis: oxidative stress, inflammation, endothelial dysfunction, and tissue remodelling.

The functional effects of five of the six RAAS polymorphisms have been studied previously. The AGT polymorphism was associated with elevated plasma angiotensinogen levels and CHD, the ACE polymorphism with elevated serum and tissue ACE levels and ischaemic heart disease. The CYP11B2 polymorphism was found to be associated with plasma renin activity and cardiovascular disease, and the ADD1 polymorphism with greater sensitivity to changes in sodium balance and cardiovascular disease in hypertensive individuals. Furthermore, the GNB3 polymorphism, which leads to a splice variant, was shown to enhance intracellular signalling with a potential to affect vascular reactivity and was associated with the risk of CHD. Since G protein-coupled receptors represent the final common pathway in both the RAAS and the adrenalin/noradrenalin system, the GNB3 polymorphism was included in both systems. The AGTR1 polymorphism was shown to be associated with CHD in FH patients and was therefore included in the present study.

The RAAS gene-load score was clearly associated with CHD, but the gene-load score of the adrenalin/noradrenalin system was not associated with CHD. An explanation for this could be that we studied these systems in the setting of severe hypercholesterolaemia. Several lines of evidence suggest that there is an interaction between hypercholesterolaemia and RAAS in the development of atherosclerosis, in which hypercholesterolaemia influences RAAS activity. For instance, it has been shown that LDL cholesterol affects the expression of the angiotensin II type 1 receptor. Although it has been suggested that the plasma noradrenalin concentrations are increased in FH, evidence of an interaction of the adrenalin/noradrenalin system and hypercholesterolaemia in the development of atherosclerosis is lacking. We investigated whether there was an interaction between LDL cholesterol and the gene-load scores. We did not find such interactions (data not shown). Notably, higher levels of total and LDL cholesterol were associated with a lower cumulative CHD risk (Table 1). An explanation for this paradoxical effect could be...
that FH patients with total and/or LDL cholesterol levels above the median received cholesterol-lowering therapy at a younger age than patients with levels below the median (42.2 vs. 45.0 years, respectively, \( p < 0.001 \), data not shown).

The strength of our study is determined by the availability of a large, well-phenotyped study population consisting of FH patients who have a severely elevated risk of CHD. However, the effect of any single gene on the susceptibility to a complex disease is likely to be modest and the pathways studied are highly redundant systems. Therefore, we investigated the association between the combined effect of genetic variants and CHD in our population with its corresponding high power to detect potentially small but accumulating effects of the genetic variants. Nevertheless, our findings need to be validated in independent FH populations. In the present study, we did not adjust for multiple testing, but it is important to note that even a Bonferroni correction would not have changed the association between the gene-load scores and CHD.

Our study has a number of limitations. First, it was a retrospective cohort study whose primary source of data was from medical records. Nonetheless, the primary endpoint CHD was well defined by using guidelines, which were developed before the data collection of this population. Secondly, our findings may solely apply to people with FH. Thirdly, the ADD1 polymorphism was not in Hardy–Weinberg equilibrium. This deviation is probably not based on mixed ethnic groups, because >99% of our FH population is Caucasian and we excluded apparent relatives. Because the polymorphism was in Hardy–Weinberg equilibrium in the FH patients with CHD, no apparent selection has taken place on the basis of CHD status. Most likely, therefore, this deviation from Hardy–Weinberg equilibrium was found by chance. It seems unlikely that genotyping errors are the cause; <0.5% of discordant results were found in a re-analysis of several polymorphisms in the original genotyping assay. Finally, the association between the RAAS gene-load score and CHD suggests an additive effect of the underlying risk genotypes. However, we cannot rule out interactions between the risk genotypes. We analysed fully unlinked loci on separate chromosomes, but the size of our population is too small to investigate all potentially functional interactions between the polymorphisms.

In conclusion, we have shown that genetic variation in the RAAS contributes to CHD risk in patients with FH in a gene-dose dependent way. Pathway-specific gene-load scores, similar to that of RAAS, may offer opportunities for the development of improved individual CHD risk assessments in patients with FH.

Supplementary material

Supplementary material is available at European Heart Journal online.

Conflict of interest: none declared.

Funding

This work has been partly funded by the Netherlands Heart Foundation (2006B190) and by Merck, Sharp and Dohme – The Netherlands.

References


