UGT1A1*28 Allele and Coronary Heart Disease: The Rotterdam Study, Piter J. Bosma, Irene M. van der Meer, Conny T. Bakker, Albert Hofman, Marianne Paul-Abramase, and Jacqueline C. Witteman (Liver Center, Academic Medical Center, Meibergdreef 9, 1105AZ Amsterdam, The Netherlands; Department of Epidemiology & Biostatistics, Erasmus MC, University Medical Center, Rotterdam, PO Box 1738, 3000DR Rotterdam, The Netherlands; author for correspondence: e-mail P.J.Bosma@amc.uva.nl)

Oxidative reactions, such as lipid oxidation and the formation of oxygen radicals, are involved in the pathophysiology of atherosclerosis and coronary heart disease (CHD). Bilirubin, the metabolic waste product of heme degradation, is an endogenous antioxidant and could thus have a protective effect against CHD. In vitro, conjugated and unconjugated bilirubin are scavengers of peroxyl radicals and are able to protect human LDL against peroxidation. In vivo, increased serum bilirubin was shown to produce an enhanced antioxidant status of serum. In addition, an association between low serum bilirubin and the risk of CHD has been observed in several studies.

A recent family heart study inferred the existence of a major gene for high bilirubin concentrations that may protect against CHD. A likely candidate gene responsible for high serum bilirubin is the gene encoding the hepatic enzyme bilirubin UDP-glucuronosyltransferase (UGT1A1). Glucuronidation is an essential step in the biliary excretion of bilirubin, and decreased UGT1A1 activity leads to increased serum concentrations of unconjugated bilirubin. A common etiology of decreased UGT1A1 activity is the insertion of a TA in the TATA box in the promoter region of the UGT1A1 gene. Transcription of the UGT1A1 gene is fivefold lower in individuals with this allele, designated UGT1A1*28.

Individuals homozygous for the UGT1A1*28 allele (genotype 7/7) have mildly increased serum bilirubin compared with heterozygotes (genotype 6/7) and homozygous wild-type individuals (genotype 6/6). In Caucasian populations, the frequency of individuals homozygous for this allele is 10–16%. This is close to the 12% for the inferred major described in the family study.

Our aim was to investigate whether the UGT1A1*28 allele is indeed responsible for the association of plasma bilirubin with CHD. Because individuals homozygous for UGT1A1*28 (7/7) have higher serum bilirubin, we hypothesized that they would have a reduced risk of CHD. Within the Rotterdam Study, a population-based cohort study in the elderly, we investigated whether the risk of myocardial infarction is associated with the presence of UGT1A1*28.

The Rotterdam Study is a large, population-based cohort study composed of 7983 men and women. Its aim is to investigate the incidence of and risk factors for chronic disabling diseases. From March 1990 until July 1993, all inhabitants of a suburb of the city of Rotterdam 55 years and older were invited to participate in an extensive home interview and two visits to the research center. The overall response rate was 78%. Written informed consent was obtained from all participants.

For the present study, individuals were classified as cases if they had experienced a myocardial infarction after their initial visit to the research center. Events were reported by general practitioners and verified by two research physicians, who independently examined patient records from the general practitioner and, in case of hospital admission, hospital records. Finally, a cardiovascular disease expert verified all events. Of the 347 cases of myocardial infarction that occurred in the Rotterdam Study until January 1, 1999, DNA was available for 239. The UGT1A1 genotype could be determined in 213 cases. Each case was matched according to age and gender with two controls. For the controls of 28 cases there was no DNA available, yielding a final selection of 185 cases presented in this study. We tested whether age, gender, smoking habits, body mass index, presence of diabetes mellitus, systolic blood pressure, total cholesterol, and HDL-cholesterol were similar in the total case population and the 185 selected cases. The 185 cases were younger [mean (SD) age, 69.6 (7.4) years] than the total case population [72.4 (8.5) years], but all other cardiovascular risk factors were comparable in both groups. We therefore consider the selected cases to be a random sample of the total case population.

Nonfasting serum total bilirubin was measured with use of a diazo-coupling method with 2,5-dichlorophenyl diazonium (Vitalab Selectro; Merck). The UGT1A1 promoter region was genotyped by real-time PCR with specific hybridization probes.

Hardy–Weinberg equilibrium was tested by a $\chi^2$ test with 1 degree of freedom. For each genotype, the mean bilirubin concentration was computed. A test for trend was performed by linear regression analysis. Odds ratios for myocardial infarction for the 6/7 and 7/7 genotypes were computed by conditional logistic regression analysis, with the 6/6 genotype as the referent category. In the present study, we had the power to detect an odds ratio of 0.56 or lower for the heterozygous and homozygous carriers of the 7 allele combined with the 6/6 genotype as the reference. All analyses were done with SPSS 9.0 for Windows.

The mean (SD) age was 69.6 (7.4) years in the cases and 68.8 (7.3) years in the controls; 61.6% of the cases and 62.0% of the controls were men. As expected, cases had significantly higher serum concentrations of total cholesterol and lower HDL-cholesterol than the controls; in the cases, there were more current smokers and individuals with diabetes mellitus. In this study, serum bilirubin concentrations were available for 114 cases and 162 controls. Serum bilirubin fluctuates during the day and is highest in the morning after an overnight fast. Perhaps because we had not collected blood samples at a standardized time, the effect of the 7/7 genotype on serum bilirubin was not as pronounced as reported in a larger group of Caucasians.
serum bilirubin clearly increased with the presence of one or two 7 alleles (P < 0.001 for trend; see Table 1). Serum bilirubin was not associated with myocardial infarction: the age- and gender-adjusted odds ratio of myocardial infarction associated with an increase in bilirubin of 1 μmol/L was 0.96 (95% confidence interval, 0.90–1.03).

The frequencies of the UGT1A1 genotypes are presented in Table 1. Controls were in Hardy–Weinberg equilibrium (P = 0.80). The risk of myocardial infarction for the 6/7 genotype compared with the 6/6 genotype was 0.9 (95% confidence interval, 0.7–1.3). The risk of myocardial infarction for the 7/7 genotype was 1.3 (0.8–2.2). After adjustment for age, gender, smoking habits and pack-years of smoking, body mass index, diabetes mellitus, systolic and diastolic blood pressure, total cholesterol, and HDL-cholesterol.

$P$ for trend $<0.001$ for trend; see Table 1. Serum bilirubin concentrations were available for 114 cases and 162 controls.

OR, odds ratio; CI, confidence interval.

Adjusted for age and gender.

Adjusted for age, gender, smoking habits and pack-years of smoking, body mass index, diabetes mellitus, systolic and diastolic blood pressure, total cholesterol, and HDL-cholesterol.

*a* Referent category.

### Table 1. Odds ratios for CHD for the UGT1A1 genotypes.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (n = 185)</th>
<th>Controls (n = 255)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Mean (SD) bilirubin, μmol/L</td>
</tr>
<tr>
<td>6/6</td>
<td>99 (53.5%)</td>
<td>7.8 (2.4)</td>
</tr>
<tr>
<td>6/7</td>
<td>63 (34.1%)</td>
<td>9.2 (3.1)</td>
</tr>
<tr>
<td>7/7</td>
<td>23 (12.4%)</td>
<td>14.8 (5.0)</td>
</tr>
</tbody>
</table>

References