Interleukin 6 – 174 G/C Promoter Polymorphism and Risk of Coronary Heart Disease

Results from the Rotterdam Study and a Meta-Analysis

Mark P.S. Sie, Fakhredin A. Sayed-Tabatabaei, Hok-Hay S. Oei, André G. Uitterlinden, Hubert A.P. Pols, Albert Hofman, Cornelia M. van Duijn, Jacqueline C.M. Witteman

Objective—Inflammation plays a pivotal role in the pathogenesis of atherosclerosis. Interleukin (IL) 6 has many inflammatory functions, and the IL-6 – 174 G/C promoter polymorphism appears to influence IL-6 levels. Findings of previous studies on the relation between this polymorphism and risk of cardiovascular diseases are inconsistent. We investigated this polymorphism in relation to risk of coronary heart disease (CHD) in a population-based study and meta-analysis.

Methods and Results—Participants (6434) of the Rotterdam Study were genotyped. Analyses on the relation between genotype and CHD were performed using Cox proportional hazards tests, and the association between genotype and plasma levels of IL-6 and C-reactive protein (CRP) was investigated. All of the analyses were adjusted for age, sex, and common cardiovascular risk factors. A meta-analysis was performed, using a random effects model. No association between genotype and risk of CHD was observed. The polymorphism was not associated with IL-6 levels, but the C-allele was associated with higher CRP levels (P < 0.01). Our meta-analysis did not show a significant association between the genotype and risk of CHD.

Conclusions—We conclude that the polymorphism is not a suitable genetic marker for increased risk of CHD in subjects ≥55 years of age. (Arterioscler Thromb Vasc Biol. 2006;26:212-217.)

Key Words: coronary heart disease ■ inflammation ■ IL-6 ■ polymorphism

Inflammatory processes play a pivotal role in the pathogenesis of atherosclerosis. Within the inflammatory pathway, cytokines fulfill a multitude of functions. Interleukin (IL) 6 is a pleiotropic inflammatory cytokine. It plays an important part in the acute-phase response and inflammatory cascade, such as upregulation of acute-phase proteins as C-reactive protein (CRP). CRP levels have been found to be associated with risk of coronary heart disease (CHD). Several studies have also shown an association between IL-6 plasma levels and cardiovascular pathology. Ridker et al found elevated IL-6 levels to be associated with increased risk of myocardial infarction (MI). This finding was replicated in another large population by Bennet et al, who found an increased risk of MI for those in the upper quartile levels of IL-6 versus those in the lowest quartile levels, and by Cesari et al, when comparing subjects with highest and lowest IL-6 tertiles in an American population. Fishman et al detected a functional polymorphism (G > C) in the promoter region of the human IL-6 gene (174 bp upstream from the start site). This polymorphism appears to influence the transcription of the IL-6 gene and also plasma levels of IL-6, and IL-6 is, therefore, a candidate gene for additional study into its role in cardiovascular disease. However, results from previous studies on the – 174 G/C polymorphism and CHD were inconsistent. Five studies, most of which were case-control studies, conducted in Western, mainly white, populations, found the C-allele to be associated with (an increased risk of) CHD or cardiovascular disease. Four other studies, however, did not find a significant association. We studied the IL-6 – 174 G/C polymorphism in relation to risk of CHD in a large population–based study. Also, a meta-analysis was conducted including studies on this polymorphism and CHD.

Methods

Study Population

The Rotterdam Study is an ongoing prospective cohort study including 7983 participants of ≥55 years in age. Its general aims are to investigate determinants of chronic diseases. During the first phase of this study (1990–1993), all of the inhabitants of a Rotterdam suburban area (Ommoord) aged ≥55 years were invited to participate in this study. The response rate was 78%. Baseline investigations included an interview and visits to the research center, where a number of clinical measurements were performed. Approval
of the Medical Ethics Committee of the Erasmus University in Rotterdam was obtained for the Rotterdam Study. From all of the participants, written informed consent was acquired. A more in-depth description of the Rotterdam Study was published previously.27

Clinical Characteristics
Trained investigators collected information using a computerized questionnaire during the home visits. The information included current health status, medical history, use of medication, and smoking behavior. During the 2 subsequent visits to the center, blood samples were obtained, and established cardiovascular risk factors were measured as described elsewhere.28 Hypertension was defined as a systolic blood pressure (SBP) of ≥160 mm Hg and/or a diastolic blood pressure of ≥100 mm Hg and/or use of antihypertensive medication (with indication hypertension). Diabetes mellitus (DM) was defined as a nonfasting serum glucose level of ≥11.1 mmol/L and/or use of antidiabetic medication. A 12-lead ECG was recorded and analyzed by the Modular ECG Analysis System.29 Evaluation of the atherosclerotic status of participants was accomplished using ultrasonography (carotid arteries), radiographic detection (aorta), and ankle-foot index (via blood pressure measurements); these methods have been extensively described previously.30

Measurement of IL-6 and CRP plasma levels
A venapuncture was performed by application of minimal stasis with a 21-gauge Butterfly needle with tube (Surflo winged infusion set, Terumo). Nonfasting blood was collected in tubes containing 0.129 mol/L sodium citrate at 4°C. The ratio of blood:sodium citrate was 9:1. Plasma was collected after centrifugation for 10 minutes at 3000 rpm. Subsequently, platelet-free plasma was obtained by centrifugation for 10 minutes at 10,000 rpm, immediately frozen in liquid nitrogen (LN2), and stored at −20°C. All of the tubes were stored on ice before and after blood sampling. Levels of IL-6 were measured by using a commercially available ELISA (Quantikine HS from R&D Systems Europe). CRP was measured in samples stored at −20°C by sensitive immunologic methods based on rate near-infrared particle immunoassays (IMMAGE from Beckman Coulter Netherlands).

Genotyping
Genotyping of the IL-6 −174 G/C polymorphism was performed using samples stored earlier at −80°C. DNA was isolated using standard procedures. Genotypes were determined in 2-ng genomic DNA with the Taqman allelic discrimination assay (Applied Biosystems, Primer Express) and stored at −80°C. All of the tubes were stored on ice before and after blood sampling. Levels of IL-6 were measured by using a commercially available ELISA (Quantikine HS from R&D Systems Europe). CRP was measured in samples stored at −20°C by sensitive immunologic methods based on rate near-infrared particle immunoassays (IMMAGE from Beckman Coulter Netherlands).

Follow-Up Procedures and Definition of Events
General practitioners (GPs) in the research district, with whom 85% of the participants of the Rotterdam Study were enlisted, reported fatal and nonfatal cardiovascular events. Research assistants verified all of the information by checking medical records at the GP offices. All of the medical records of the participants under the care of GPs were checked annually for possible events. Letters and, in case of hospitalization, discharge reports from medical specialists were obtained. With respect to the vital status of participants, information was also obtained regularly from the municipal health authorities in Rotterdam. After notification, the cause and circumstances of death were established by a questionnaire from the GPs. Two research physicians independently coded all of the reported events according to the International Classification of Diseases, 10th Edition.31 Codes on which the research physicians disagreed were discussed to reach consensus. Finally, a medical expert in cardiovascular disease, whose judgment was considered final, reviewed all of the events. CHD was defined (based on International Classification of Diseases, 10th Edition) as the occurrence of an MI (121), revascularization procedure (percutaneous transluminal coronary angioplasty or coronary artery bypass graft; Z95.5 and Z95.1), ischemic heart disease (I20 and I22 through I25), sudden cardiac death (I46), ventricular fibrillation or tachycardia (I49), congestive heart failure (I50), or sudden death undefined (R96) during follow-up. Sudden death was defined as death occurring instantaneously or within 1 hour after the onset of symptoms. Incident MI was defined as the occurrence of a fatal or nonfatal MI after the baseline examination.

Meta-Analysis
For the meta-analysis, published data were used from previously published studies, concerning the IL-6 −174 G/C polymorphism and CHD, until December 2004. In addition, our own data were also included. Studies were found with Pubmed/Medline using the following key words: IL-6, −174, polymorphism, cardiovascular disease, MI, and CHD and using references from retrieved articles. Eight studies were identified, of which 7 were included in the analysis.15,19–23 One study was excluded, because no genotype frequencies were available specified for cases and controls.24 All of the studies were conducted in European populations. As end point in the analyses, we used CHD, defined as the occurrence of an MI, revascularization procedure (percutaneous transluminal coronary angioplasty or coronary artery bypass graft), ischemic heart disease, (sudden) cardiac death, ventricular fibrillation or tachycardia, sudden death undefined, or congestive heart failure.

Statistical Analyses
Baseline characteristics were tested for differences between the genotypes using ANOVA analyses for continuous variables [age, body mass index (BMI), SBP, cholesterol levels, IL-6 levels, and CRP levels] and Pearson χ2 tests for discrete variables (sex, smoking, diabetic status, and history of MI). All of the values above mean plus 3 times the SD were excluded, as correction for outliers. Natural-log transformed values of IL-6 and CRP levels were used to normalize the distribution of these variables; presented data are back transformed. Cox proportional hazards analyses were performed to obtain relative risks. All of the analyses were adjusted for age and sex and, additionally, for BMI, SBP, high-density lipoprotein and total cholesterol levels, baseline smoking, and DM. Subjects with prevalent MI were excluded from the analyses. Additional analyses were performed in various age strata (10-year strata starting from 55 years, and above and below 75 years), in strata of sex, smoking, and DM, and in various strata of atherosclerosis (tertiles of aorta calcification and above and below 75 years), in strata of sex, smoking, and DM, and in various strata of atherosclerosis (tertiles of aorta calcification and above and below 0.9). A P value of ≤0.05 was considered significant in all of the analyses. The statistical analyses were performed using SPSS 11 and S-Plus 6.0 for MS-Windows.

For the meta-analysis, the method of moments has been used to calculate the relative risks in a random-effects model for the pooled data.32 We used the funnel plots to examine publication bias of reported associations. The meta-analysis and heterogeneity analysis were performed using Review Manager 4.2.7 (RevMan Analyses version 1.0; Cochrane Collaboration–Wintertree Software Inc).

Results
Rotterdam Study Results
Genotyping of the IL-6 −174 G/C polymorphism was successfully performed in 6434 subjects. The major characteristics of the study population are summarized in Table 1 (2612 male and 3822 female). Mean age was 69.5 (±9.1) years. There were 2301 participants (36%) with the GG genotype (wild-type), 3050 (47%) with the GC genotype, and 1083 (17%) with the CC genotype. Genotype and allele proportions were in Hardy Weinberg equilibrium (P=0.18).

IL-6 plasma levels were determined in a random subgroup of 641 subjects after exclusion of outliers (2%). No signifi-
cant difference in mean IL-6 plasma levels between the genotypes was observed (Figure 1). CRP plasma levels were successfully determined in 5924 cases after exclusion of outliers (1%). The level of CRP was significantly higher in CC (P<0.01) and GC (P<0.01) individuals as compared with the individuals with the wild-type genotype GG (Figure 1). The C-allele was significantly associated with higher CRP levels (P<0.01).

During a mean follow-up period of 6.8 (± 2.3) years, there were 648 cases of CHD and 280 cases of MI. After exclusion of patients with previous MI, there were 463 newly diagnosed cases of CHD and 208 cases of incident MI.

There was no significant difference in relative risk of CHD or MI when comparing the GC or CC genotypes and C-allele carriership with the GG wild-type genotype (P<0.01). Adjusting for age, BMI, SBP, total and high-density lipoprotein cholesterol, smoking, and DM did not alter these results.

No difference in survival and event-free survival was found among the genotypes CC, GC, and GG for CHD in men (P=0.36) or women (P=0.79). There was also no difference in survival between the genotypes for MI in men (P=0.81) or women (P=0.63). Additional adjustment for covariates did not alter these findings. No significant associations between the −174 genotype and the risk of CHD or MI were observed in strata of age, smoking, diabetic status, and levels of atherosclerosis (data not shown).

Meta-Analysis Results
In the meta-analysis were a total of 13 434 C-allele carriers of whom 36% (n=4799) were CHD cases and 6364 subjects with the wild-type GG genotype of whom 33% (n=2128) were CHD cases. The meta-analysis did not show a significant association between the polymorphism and CHD. Subjects with the CC genotype compared with individuals with the GG genotype had a relative risk of 1.03 (95% CI, 0.92 to 1.16; P=0.59). Also when carriers of the C-allele were compared with the GG genotype, there was no significant difference in risk, 1.12 (95% CI, 0.97 to 1.29; P=0.12; Figure 2). There was evidence for heterogeneity (P=0.003). This was caused by the study of Licastro et al,24 which was an outlier with a very high odds ratio (Figures 2 and 3). We did not exclude this study, because the exclusion would only shift the nonsignificant results further toward the null value and would not lead to a significant difference in the overall outcome.

Discussion
In this prospective population-based study, no significant association between the IL-6 −174 G/C genotype and CHD or MI was found, nor was there an association with plasma levels of IL-6. A higher CRP level was found among subjects with the C-allele. Adjusting for possible confounders and

TABLE 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall</th>
<th>IL-6 −174 Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG (n=2301)</td>
<td>GC (n=1050)</td>
</tr>
<tr>
<td>No. (n, %)</td>
<td>2301 (36)</td>
<td>1050 (47)</td>
</tr>
<tr>
<td>Age, y</td>
<td>69.2 ± 0.9</td>
<td>69.5 ± 0.9</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>958 (42)</td>
<td>1234 (41)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.1 ± 3.7</td>
<td>26.4 ± 3.8</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>139 ± 22.3</td>
<td>139 ± 22.3</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.6 ± 1.2</td>
<td>6.8 ± 1.2</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.4 ± 0.4</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>Smoking, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>506 (22)</td>
<td>696 (23)</td>
</tr>
<tr>
<td>Former</td>
<td>950 (41)</td>
<td>1223 (40)</td>
</tr>
<tr>
<td>Never</td>
<td>781 (34)</td>
<td>1044 (34)</td>
</tr>
<tr>
<td>Diabetes, no. (%)</td>
<td>222 (10)</td>
<td>333 (11)</td>
</tr>
<tr>
<td>History of MI, no. (%)</td>
<td>255 (11)</td>
<td>377 (12)</td>
</tr>
</tbody>
</table>

*GC different from GG (P<0.04), †Significantly different from GG (P<0.01), ‡Significantly different from GG (P<0.01).

Figure 1. *Significantly different from GG (P<0.01). †Significantly different from GG (P<0.01).
analyses in various substrata did not alter these findings. A meta-analysis, including our own study, showed no significant association between the genotype and risk of CHD.

Our study is based on a large, ongoing population-based study in a relatively homogeneous population, because 98% of the participants in our study are white and are all living in the same area, a suburb of Rotterdam. In contrast to case control studies, the prospective nature of our study makes our results less prone to survival bias. We adjusted all of the analyses for established risk factors, but this did not change the estimates.

Results from previous studies on this IL-6 \(^{174}G/C\) polymorphism and CHD were inconsistent (Table I, available online at http://atvb.ahajournals.org).\(^{15,19–26}\) In the Ludwigshafen Risk and Cardiovascular Health (LURIC) cohort and several other studies, no association between this polymorphism and CHD was found.\(^{15,19,20,25}\) Other studies, such as the Etude Cas-Temoins de l’Infarctus du Myocarde (EC-TIM) Study and a study based on the CHS cohort, found a higher risk of CHD associated with the C-allele.\(^{21–24,26}\) All of the studies on IL-6 genotype and CHD were performed in Western populations with predominantly male subjects with an average age of \(\geq 50\) years. It is, therefore, unlikely that differences in findings are because of ethnicity, gender, or age differences between the studies.

To provide a better overview of (inconsistent) findings of various studies, we also studied the association between the IL-6 genotype and the risk of CHD by performing a meta-analysis, which made it possible to study a very large number of events. This meta-analysis also did not show an association between the genotype and risk of CHD. Publication bias is always an important potential source of bias in meta-analyses. However, studies have been published both with positive and negative findings on the association between the polymorphism and the risk of CHD, and the funnel plot does not suggest a strong publication bias.

There is also no consensus on the effect of the genotype on plasma levels of IL-6 and CRP.\(^{14,15,19–21,23,25,26}\) The C-allele in our study was significantly associated with higher CRP levels. In most studies showing an effect of the C-allele on plasma levels, the C-allele was associated with higher plasma levels of both IL-6 and CRP.\(^{20,21,26,33}\) This is pathophysiologically plausible, because CRP is produced in the liver, and IL-6 is a hepatocyte stimulant, so elevated IL-6 levels will result in higher CRP levels. However, IL-6 has been described to be too unstable in time (plasma half-life of \(<2\) hours) to be measured precisely.\(^{24,35}\) In addition, there was a limited sample size of IL-6 levels. Given the short half-life, larger numbers would have been needed to detect a relatively small difference in IL-6 levels. This might explain the lack of

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CHD Events/Total</th>
<th>RR</th>
<th>95% CI</th>
<th>P Value</th>
<th>MI Events/Total</th>
<th>RR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>GG*</td>
<td>84/801</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>44/801</td>
<td>1</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>GC</td>
<td>116/1019</td>
<td>1.10</td>
<td>0.83 to 1.46</td>
<td>0.49</td>
<td>54/1019</td>
<td>0.96</td>
<td>0.65 to 1.44</td>
<td>0.86</td>
</tr>
<tr>
<td>CC</td>
<td>43/352</td>
<td>1.19</td>
<td>0.83 to 1.72</td>
<td>0.35</td>
<td>21/352</td>
<td>1.10</td>
<td>0.66 to 1.86</td>
<td>0.71</td>
</tr>
<tr>
<td>Carrier</td>
<td>159/1371</td>
<td>1.13</td>
<td>0.86 to 1.47</td>
<td>0.38</td>
<td>75/1371</td>
<td>1.00</td>
<td>0.69 to 1.45</td>
<td>1.00</td>
</tr>
<tr>
<td>Females</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG*</td>
<td>74/1245</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>29/1245</td>
<td>1</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>GC</td>
<td>106/1654</td>
<td>1.05</td>
<td>0.78 to 1.41</td>
<td>0.76</td>
<td>43/1654</td>
<td>1.09</td>
<td>0.68 to 1.75</td>
<td>0.71</td>
</tr>
<tr>
<td>CC</td>
<td>40/613</td>
<td>1.02</td>
<td>0.70 to 1.50</td>
<td>0.92</td>
<td>17/613</td>
<td>1.14</td>
<td>2.08 to 0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>Carrier</td>
<td>146/2267</td>
<td>1.04</td>
<td>0.79 to 1.38</td>
<td>0.78</td>
<td>60/2267</td>
<td>1.11</td>
<td>0.71 to 1.72</td>
<td>0.66</td>
</tr>
</tbody>
</table>

RR, relative risk. Data are age adjusted.

*Reference genotype.

Figure 2. Meta-analysis of CHD risk for carriers of the C-allele vs the GG genotype.
association between the genotype and IL-6 plasma levels in our study.

In our study, we did not find a clear association between genotype and risk of CHD. This may be related to the fact that the functionality of the polymorphism, at least with respect to the extent of the influence on IL-6 plasma levels, has not been definitively established. Although an effect on transcription and IL-6 levels was described, the view presented by Terry et al., whereby the effect is cell-specific and dependent on complex interactions between several polymorphisms, rather than on an individual polymorphism, might be more applicable. This implies that the solitary −174 G/C genotype might influence plasma levels but not in a substantial way.

There is evidence for the IL-6 −174 G/C polymorphism to be in linkage disequilibrium with other functional but less frequently investigated polymorphisms, such as −597 G/A and −572 G/C, and with possibly functional polymorphisms in the −373 AT run. Because of this linkage disequilibrium, these polymorphisms were not considered in our analyses, as analyzing these would provide similar information.

In conclusion, we did not find a significant relation between the IL-6 promoter polymorphism −174 G/C and risk of CHD. Based on our analyses and results from our meta-analysis, we conclude that the polymorphism does not have a prominent role in the pathogenesis of CHD and is, therefore, not a suitable genetic marker for increased risk of CHD.

Acknowledgments

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References


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<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Georges et al., 2001 (ref. 22)</td>
<td>Cases: 640 Controls: 719 Population: UK, France – males Age: 25-64 yrs</td>
<td>1.0 * 1.35 † (1.05-1.73) 1.31 (0.94-1.84)</td>
</tr>
<tr>
<td>Humphries et al., 2001 (ref. 21)</td>
<td>Follow-up: 2751 (2560 cases and 160 controls) Population: UK – males Age (mean): 57 yrs (cases) / 56 yrs (controls)</td>
<td>1.0 * 1.54 † (1.06-2.22) 1.11 (0.67-1.83)</td>
</tr>
<tr>
<td>Basso et al., 2002 (ref. 20)</td>
<td>Cases: 498 Controls: 1109 Population: Scotland – males Age (mean): 56 yrs (cases &amp; controls)</td>
<td>1.0 * 1.07 (0.77-1.48) 1.26 (0.83-1.91)</td>
</tr>
<tr>
<td>Nauck et al., 2002. (ref. 19)</td>
<td>Cases: 2559 Controls: 729 Population: Germany – males &amp; females Age (mean): 64 yrs (cases) / 58 yrs (controls)</td>
<td>1.0 * 0.98 (0.79-1.16) 0.95 (0.75-1.20)</td>
</tr>
<tr>
<td>Jenny et al., 2002 (ref. 23)</td>
<td>Cases: 770 + 250 Controls: 500 Population: USA (Caucasians + African) – males &amp; females Age (mean): 73 yrs</td>
<td>1.0 * 1.50 † (1.05-2.14)</td>
</tr>
<tr>
<td>Bennet et al., 2003 (ref. 15)</td>
<td>Cases: 1179 Controls: 1528 Population: Sweden – males &amp; females Age (mean): 59 yrs (males, cases &amp; controls)</td>
<td>1.0 * - 1.1 ‡ (0.8-1.4)</td>
</tr>
<tr>
<td>Licastro et al., 2004 (ref. 24)</td>
<td>Cases: 139 Controls: 198 Population: Italy - males Age (mean): 65 yrs (cases) / 57 yrs (controls)</td>
<td>1.0 * 2.65 † (1.53-4.62)</td>
</tr>
<tr>
<td>Lieb et al., 2004 (ref. 25)</td>
<td>Cases: 1322 Controls: 1023 Population: Germany – males &amp; females Age (mean): 58 yrs &amp; 56 yrs (2 case groups) / 52 yrs (controls)</td>
<td>1.0 * 0.92 § (0.78-1.10)</td>
</tr>
</tbody>
</table>

* reference, † significant, ‡ men, § women, || not provided in original article; the relative risk mentioned is derived from our meta-analysis