Genetic and Environmental Influences on the Prospective Correlation Between Systemic Inflammation and Coronary Heart Disease Death in Male Twins

Sheng-Hui Wu, Michael C. Neale, Anthony J. Acton Jr, Robert V. Considine, Ruth E. Krasnow, Terry Reed, Jun Dai

Objective—Because of lack of evidence, we aimed to examine to what degree low-grade systemic inflammation and coronary heart disease (CHD) death shared common genetic and environmental substrates.

Approach and Results—From the 41-year prospective National Heart, Lung, and Blood Institute Twin Study, we included 950 middle-aged male twins at baseline (1969–1973). Low-grade systemic inflammation was measured with plasma levels of interleukin-6 (IL-6) and C-reactive protein. Univariate and bivariate structural equation models were used, adjusted for a risk score for CHD death. The score-adjusted heritability was 19% for IL-6, 27% for C-reactive protein, and 22% for CHD death. The positive phenotypic correlation of IL-6 with CHD death (r\text{adjusted}=0.27; 95% confidence interval [CI], 0.08–0.43) was driven by additive genetic factors (contribution [relative contribution], 0.30 [111%]) but attenuated by unique environment (−0.03 [−11%]). The genetic correlation between IL-6 and CHD death was 0.74 (95% CI, 0.21–1.00), whereas the unique environmental correlation was −0.05 (95% CI, −0.35 to 0.25). The proportion of genetic variance for CHD death shared with that for IL-6 was 74%. The phenotypic correlation of C-reactive protein with CHD death (r\text{adj}=0.10; 95% CI, −0.02 to 0.22) was explained by additive genetic factors (0.20 [149%]) but was attenuated by the unique environment (−0.09 [−49%]). The genetic correlation of C-reactive protein with CHD death was 0.63 (95% CI, −0.07 to 1.00), whereas the unique environmental correlation was −0.07 (95% CI, −0.29 to 0.17).

Conclusions—Low-grade systemic inflammation, measured by IL-6, and long-term CHD death share moderate genetic substrates that augment both traits. (Arterioscler Thromb Vasc Biol. 2014;34:00-00.)

Key Words: C-reactive protein • interleukin-6 • mortality • twins

The role of the immune system and inflammatory pathways in the development of atherosclerotic disease is well established. Circulating interleukin-6 (IL-6) and C-reactive protein (CRP) are biomarkers for systemic inflammation and are positively associated with coronary heart disease (CHD) risk. IL-6 is a proinflammatory cytokine, which stimulates hepatic production of CRP as an acute-phase protein, modulates cell adhesion, and promotes coagulation of platelets. CRP may directly interact with atherosclerotic vessels or ischemic myocardium by activation of the complement system, thereby promoting inflammation and thrombosis. Genetic factors contribute to plasma levels of IL-6 and CRP and CHD mortality. However, no previously published studies have explored the degree to which the correlation of IL-6 or CRP with CHD death is due to genetic or environmental factors.

We used a classical twin design with inclusion of monozygotic twins (MZ) and dizygotic twins (DZ) reared together to address this unclear area. MZ have identical genomes, whereas DZ share on average half of their genomes. If genetic factors influence a trait, MZ should be more similar than DZ. By comparing the similarity between MZ and DZ, we can quantify genetic and environmental contribution to traits. When 2 traits like inflammation and CHD death are studied, each trait can be influenced by its own set of genetic and environmental factors. The overlapping of the 2 sets of genetic and environmental factors may exist. A statistical model—bivariate Cholesky decomposition model—is used to assess if 2 traits are phenotypically correlated and the extent that these factors for the 2 traits are overlapped. For example, a prior twin study found that white matter hyperintensities were correlated with physical function with a phenotypic correlation of −0.20. The genetic factors for white matter hyperintensities and executive function were overlapped because the genetic correlation coefficient (r) was −0.24. The negative value of this coefficient meant that the genetic factors augmenting white matter hyperintensities decreased executive function or...
vice versa. Similarly, this prior study also found that the unique environmental factors that were not shared between cotwins within a pair for each trait were overlapped because unique environmental correlation coefficient \( r_e \) was –0.22. The negative value of the \( r_e \) indicated that the unique environmental factors strengthening white matter hyperintensities weakened executive function or vice versa. In our classic twin study, we aimed to examine whether genetic and environmental factors explained the prospective correlation of IL-6 or CRP with CHD death in a population-based longitudinal study and to quantify the degree to which low-grade systemic inflammation and CHD deaths shared genetic and environmental substrates.

### Materials and Methods

Materials and Methods are available in the online-only Supplement.

### Results

#### Characteristics of the Study Population

During 41-year follow-up time, there were 143 CHD deaths (74 MZ and 69 DZ) among 950 twins (218 MZ and 227 DZ twin pairs, 31 unpaired MZ and 29 unpaired DZ twins). Twins who died of CHD had higher body mass index and diastolic and systolic blood pressure, higher plasma levels of total cholesterol and triglycerides, and a higher risk score than those not at baseline (Table 1). Plasma levels of IL-6 were higher among twins who died of CHD (Table 1). A flow diagram illustrated the conceptual framework of univariate and bivariate analyses (Figure).

#### Univariate Model Fitting

The AE model was the most parsimonious model for each of IL-6, CRP, and CHD death and was used to estimate the heritability (Table 2). The heritability was similar before and after adjustment for either baseline age or the risk score. Genetic components contributed to inflammation and CHD death. The risk score–adjusted heritability was 19\% (95\% confidence interval [CI], 2–34\%) for IL-6, 27\% (95\% CI, 10–42\%) for CRP, and 22\% (95\% CI, 0–45\%) for CHD death during a 41-year follow-up period. Unique environmental factors explained the remaining variance.

#### Bivariate Model Fitting

The AE model was the best-fitting Cholesky model for the bivariate analyses (Table 3). The unadjusted prospective phenotypic correlation between IL-6 and CHD death (0.16; 95\% CI, 0.03–0.28) was statistically significant. The age-adjusted and risk score–adjusted results were similar and were stronger than the unadjusted correlation. After adjustment for the risk score, a higher IL-6 level was significantly related to the higher CHD death risk (phenotypic correlation, 0.27; 95\% CI, 0.08–0.43). The genetic contribution was large (relative contribution, 111\%), and the unique environmental contribution was small (relative contribution, –11%; Table 3). The score-adjusted relative genetic contribution was >1, indicating that the positive part of the phenotypic correlation was driven by the genetic component, whereas the score-adjusted relative unique environmental contribution was <0, indicating that these factors contributed negatively to the adjusted phenotypic covariance.

We further examined whether the genetic component linking IL-6 to CHD death was common to both traits (Table 3). Unadjusted \( r_g \) and \( r_e \) between IL-6 and CHD death were not statistically significant. After either age or score adjustment, \( r_g \) was statistically significant, whereas \( r_e \) was negative but remained nonsignificant. Both adjustments generated similar results. After adjusting for risk score, \( r_g \) was 0.74 (95\% CI, 0.21–1.00), whereas \( r_e \) was –0.05 (95\% CI, –0.35 to 0.25; Table 3). The score-adjusted genetic correlation suggested that the genetic origin of CHD death might be shared with the genetic origin of IL-6. Approximately 74\% (ie, 0.74) of 0.22 of the heritability for CHD death (Table 2) was shared with IL-6, but 26\% (1–0.74) was independent of IL-6, supporting half of the genes influencing the 2 traits were shared. By contrast, 95\% (1–0.05) of the unique environmental origin of CHD death was independent of IL-6.

The unadjusted, age-adjusted, and score-adjusted phenotypic correlations between CRP and CHD death were not statistically significant, to which the point estimate was >1 for relative genetic contribution and <0 for relative unique environmental contribution (Table 3). Crude, age-adjusted, and score-adjusted \( r_g \) and \( r_e \) between CRP and CHD death were materially similar (Table 3). The score-adjusted genetic correlation was moderate \((r_g=0.63)\). Approximately 63\% (0.63) of 0.22 of the heritability for CHD death (Table 2) was shared with CRP, but 37\% (1–0.63) was independent of CRP, suggesting less than half of the genes influencing the 2 traits were shared. By contrast, 95\% (1–0.07) of the unique environmental origin of CHD death was independent of CRP.

In the sensitivity analysis, similar results were obtained after additional adjustment for plasma sample collection examination (Table I in the online-only Data Supplement). In another sensitivity analysis, the results did not change materially after excluding the twins with missing data on biomarkers (n=558; data not shown).

In the secondary analysis, we evaluated the degree to which low-grade systemic inflammation and CVD or all-cause mortality shared genetic and environmental factors (Table II in the online-only Data Supplement). Result patterns were similar to those between IL-6 and CHD death, except the correlation between IL-6 and all-cause mortality (Table II in the online-only Data Supplement). The score-adjusted point estimate of the relative genetic contribution to the correlation between IL-6 and all-cause mortality was 85\%, whereas the relative unique environmental contribution was 15\% (Table II in the online-only Data Supplement).

### Discussion

We found that both genetic and unique environmental factors explained variation in IL-6, CRP, and CHD death after adjustment for the composite risk score. The risk score–adjusted,
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The risk score–adjusted phenotypic correlation between CRP and CHD death was not significant and was driven by genetic factors but attenuated by unique environmental factors.

The heritability for IL-6 and CRP in our study was in the range of previously reported heritability for IL-6 (17% to 57%)12–14 and for CRP (20% to 61%).12,14,16,17 The heritability for CHD mortality in our study was lower than those in 2 prior prospective twin studies: 45% among male twins in a Danish twin study21 and 57%20 among male twins in a Swedish twin study. Our male twins were born between 1917 and 1927 and aged 58 to 94 years at death. The Danish male twins were born between 1870 and 1930 and aged 50 to 84 years at death and had a CHD mortality rate of 24%.21 The Swedish male twins were born between 1886 and 1925 and aged 36 to >85 years at death with a 46% mortality rate of CHD.20 Differences among twin populations including differences in the mortality rates of CHD,21 birth years, and age at death22 might explain differences in the heritability of CHD death among twin studies.

Our study was the first prospective cohort study to quantify overall genetic and environmental contributions to the correlation of low-grade systemic inflammation with CHD death in a temporal sequence. We found that genetic factors contributed positively to the adjusted phenotypic correlation between IL-6 and CHD death but that unique environmental factors contributed negatively. Similar phenomena were observed in a previous study of the correlation between brachial pulse pressure and aortic pulse wave velocity25 and another study of the correlation between white matter hyperintensities and physical function.24 For example, the relative genetic contribution to the phenotypic correlation between brachial pulse pressure and aortic pulse wave velocity was 1.68, common environmental contribution was –0.43, and unique environmental contribution was –0.25.25 In this scenario, environmental factors attenuated the phenotypic correlation between 2 traits due to genetic factors.

We found  \( r_g \) was greater than the phenotypic correlation coefficient for IL-6 and CRP with CHD death during the 41-year follow-up time. Similar phenomenon was found in a previous study of speech fluency, where the  \( r_g \) (0.75) was greater than the phenotypic correlation coefficient (0.72) between stuttering and nonfluency.26 The higher  \( r_g \) indicated that genes for 2 traits were overlapped to a greater extent, while the magnitude of phenotypic correlation coefficient did not measure the overlap extent. Our finding supported that genetic factors shared between IL-6 and CHD death explained their phenotypic correlation.

### Table 1. Characteristics of Twins

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Twins Died of CHD</th>
<th>Twins Not Died of CHD</th>
<th>( P ) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>143</td>
<td>807</td>
<td>...</td>
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</tbody>
</table>

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<tr>
<th>Inflammatory biomarkers</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>IL-6, median (25th, 75th percentile), pg/mL</td>
<td>2.3 (1.2, 3.5)</td>
<td>1.6 (1.1, 2.6)</td>
<td>0.02</td>
</tr>
<tr>
<td>CRP, median (25th, 75th percentile), μg/mL</td>
<td>2.5 (0.9, 6.5)</td>
<td>1.8 (1.0, 3.6)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

CHD indicates coronary heart disease; IL-6, interleukin-6; and CRP, C-reactive protein.

* \( P \) values for comparing twins who died of CHD with those not. \( P \) values were derived from mixed models for continuous variables and generalized estimating equation logistic models for dichotomous variables to account for clustering within twin pairs.

†Risk score contained information about age, smoking, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, diabetes mellitus, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.

Statistically significant, and positive phenotypic correlation between IL-6 and CHD death was driven by genetic factors but attenuated by unique environmental factors. The risk score–adjusted genetic correlation between IL-6 and CHD death was moderate, positive, and statistically significant, whereas the risk score–adjusted unique environmental correlation was weakly negative and not statistically significant.
### Table 2. Parameter Estimates From Univariate Model for Systemic Inflammation and Coronary Heart Disease Death in Twins

<table>
<thead>
<tr>
<th>Model Fitting*</th>
<th>Components of Variance Estimates†</th>
<th>Goodness-of-Fit Tests‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$a^2$ (95% CI)</td>
<td>$c^2$ (95% CI)</td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td></td>
<td></td>
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<tr>
<td>Crude model</td>
<td>ACE 0.22 (0.00–0.37) 0.00 (0.00–0.28) ... 0.78 (0.63–0.95)</td>
<td>–9.2</td>
</tr>
<tr>
<td></td>
<td>ADE 0.21 (0.00–0.37) ... 0.02 (0.00–0.38) 0.77 (0.62–0.94)</td>
<td>–9.2</td>
</tr>
<tr>
<td></td>
<td>AE§ 0.22 (0.06–0.37) ... ... 0.78 (0.63–0.94)</td>
<td>–11.2</td>
</tr>
<tr>
<td>Age-adjusted model</td>
<td>ACE 0.20 (0.00–0.35) 0.00 (0.00–0.26) ... 0.80 (0.65–0.97)</td>
<td>–18.4</td>
</tr>
<tr>
<td></td>
<td>ADE 0.16 (0.00–0.35) ... 0.03 (0.00–0.36) 0.80 (0.64–0.97)</td>
<td>–18.4</td>
</tr>
<tr>
<td></td>
<td>AE§ 0.20 (0.03–0.35) ... ... 0.80 (0.65–0.97)</td>
<td>–20.4</td>
</tr>
<tr>
<td>Score-adjusted model</td>
<td></td>
<td>ACE 0.18 (0.00–0.34) 0.00 (0.00–0.25) ... 0.82 (0.66–0.99)</td>
</tr>
<tr>
<td></td>
<td>ADE 0.08 (0.00–0.34) ... 0.12 (0.00–0.36) 0.80 (0.64–0.97)</td>
<td>–28.8</td>
</tr>
<tr>
<td></td>
<td>AE§ 0.19 (0.02–0.34) ... ... 0.81 (0.66–0.98)</td>
<td>–30.7</td>
</tr>
<tr>
<td><strong>CRP</strong></td>
<td></td>
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<tr>
<td>Crude model</td>
<td>ACE 0.15 (0.00–0.43) 0.11 (0.00–0.33) ... 0.74 (0.57–0.90) 506.6</td>
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<tr>
<td></td>
<td>ADE 0.29 (0.00–0.44) ... 0.00 (0.00–0.42) 0.71 (0.56–0.88) 505.2</td>
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<tr>
<td></td>
<td>AE§ 0.29 (0.12–0.44) ... ... 0.71 (0.56–0.88) 505.0</td>
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</tr>
<tr>
<td>Age-adjusted model</td>
<td>ACE 0.15 (0.00–0.43) 0.11 (0.00–0.33) ... 0.74 (0.57–0.90) 507.0</td>
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<td></td>
<td>ADE 0.29 (0.00–0.44) ... 0.00 (0.00–0.42) 0.71 (0.56–0.88) 507.0</td>
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<tr>
<td></td>
<td>AE§ 0.29 (0.12–0.44) ... ... 0.71 (0.56–0.88) 507.0</td>
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</tr>
<tr>
<td>Score-adjusted model</td>
<td></td>
<td>ACE 0.15 (0.00–0.42) 0.10 (0.00–0.32) ... 0.75 (0.58–0.92) 488.9</td>
</tr>
<tr>
<td></td>
<td>ADE 0.27 (0.00–0.42) ... 0.00 (0.00–0.41) 0.73 (0.58–0.91) 489.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AE§ 0.27 (0.10–0.42) ... ... 0.73 (0.58–0.90) 487.1</td>
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<tr>
<td><strong>CHD</strong></td>
<td></td>
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<tr>
<td>Crude model</td>
<td>ACE 0.22 (0.00–0.45) 0.00 (0.00–0.45) ... 0.78 (0.55–1.00)</td>
<td>–1144.7</td>
</tr>
<tr>
<td></td>
<td>ADE 0.00 (0.00–0.44) ... 0.25 (0.00–0.48) 0.75 (0.52–1.00)</td>
<td>–1145.0</td>
</tr>
<tr>
<td></td>
<td>AE§ 0.22 (0.00–0.45) ... ... 0.78 (0.55–1.00)</td>
<td>–1146.7</td>
</tr>
<tr>
<td>Age-adjusted model</td>
<td>ACE 0.22 (0.00–0.45) 0.00 (0.00–0.45) ... 0.78 (0.55–1.00)</td>
<td>–1144.7</td>
</tr>
<tr>
<td></td>
<td>ADE 0.00 (0.00–0.44) ... 0.25 (0.00–0.48) 0.75 (0.52–1.00)</td>
<td>–1141.0</td>
</tr>
<tr>
<td></td>
<td>AE§ 0.22 (0.00–0.45) ... ... 0.75 (0.55–1.00)</td>
<td>–1146.7</td>
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<tr>
<td>Score-adjusted model</td>
<td></td>
<td>ACE 0.22 (0.00–0.45) 0.00 (0.00–0.32) ... 0.78 (0.55–1.00)</td>
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<td>ADE 0.00 (0.00–0.44) ... 0.25 (0.00–0.48) 0.75 (0.52–1.00)</td>
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<td></td>
<td>AE§ 0.22 (0.00–0.45) ... ... 0.78 (0.55–1.00)</td>
<td>–1146.7</td>
</tr>
</tbody>
</table>

AIC indicates Akaike’s Information Criterion; CHD, coronary heart disease; CI, confidence interval; CRP, C-reactive protein; and IL-6, interleukin-6.

*Structural equation modeling was used. A, C, D, and E refer to additive genetic, shared environmental, dominant genetic, and unique environmental influences, respectively.

†$a^2$, $c^2$, $d^2$, and $e^2$ are estimates of the proportion of additive genetic, shared environmental, dominant genetic, and unique environmental components of variance, respectively.

‡Goodness-of-fit statistic. The lower AIC, the better the model fitting.

§Best fitting model.

||Adjustment for risk score that contained information about age, smoking, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, diabetes mellitus, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.
Our study suggested that IL-6 and CHD death shared moderate genetic substrates, which augmented both traits. However, our twin study was unable to identify specific genes that simultaneously affected IL-6 and CHD death. Among previously published genetic studies that did not identify specific genes influencing both IL-6 and CHD death, 27–30 2 studies suggested that both traits shared common genes, whereas the other 2 studies did not.28,29 A prior study of a variant of the IL-6 gene found that the −572 G>C polymorphism influenced IL-6 gene transcription and modulated plasma levels of IL-6. A meta-analysis of 27 studies found that −572 C allele was associated with a lower risk of CHD (including events and deaths) than −572 G allele.27 Identification of genes other than −572 G>C, which were common to IL-6 and CHD death, would require additional studies.

We could not rule out the existence of genetic factors shared between CRP and CHD death. Given the 95% CIs for genetic correlation between both traits, the risk score–adjusted genetic correlation could be as small as −0.07 or as large as 1.00. Prior studies of the existence of shared genes were inconsistent.32,33 In a case–control study, the single-nucleotide polymorphism rs2794521 (−717A>G) in the CRP gene promoter was independently associated with the presence of CHD among Chinese men but not with fasting serum CRP levels. Men with AG and AA genotypes had a 6.8-fold higher risk of developing CHD than those with GG genotype.32 The same single-nucleotide polymorphism was also correlated with the occurrence of myocardial infarction or thromboembolic stroke but not higher plasma levels of CRP among men in the prospective Physician’s Health Study.31 An animal experiment supported this single-nucleotide polymorphism in relation to CHD.34 In this animal study, in vitro functional experiments showed that the A allele relative to the G allele increased transcriptional activity of the promoter of the CRP gene.34

Our twin study was different from Mendelian randomization studies in the research aim and the methodology. In our twin study, plasma levels of inflammatory markers but not genetic factors were directly measured. We aimed to quantify the extent of shared genetic and environmental contributions to the relation between circulating inflammatory biomarkers and CHD. By contrast, in Mendelian randomization studies, genetic polymorphisms related to circulating inflammatory biomarker levels were directly measured as proxies for their circulating levels. The aim of Mendelian randomization studies was to evaluate the association between the circulating biomarker levels and CHD rather than understanding genetic mechanisms leading to disease.35 These studies assumed the random allocation of gene alleles at conception and were heavily built on the logic reasoning: if inflammatory biomarker levels in circulation were causally linked to CHD, genetic variants influencing the biomarker levels should also be associated with CHD.35,36 For example, a Mendelian randomization meta-analysis of 9417 coronary artery disease patients and 15982 controls aimed to test the correlation between circulating IL-6 concentrations with coronary artery disease risk.36 IL-6 gene 174C/G polymorphism was used as the proxy for circulating IL-6 levels.36 The CC/GC genotypes represented higher circulating IL-6 levels while GG represented lower levels. This was equivalent to the dichotomization of IL-6 levels. The dichotomization using the gene polymorphism might explain lack of a significant association between the 174 C/G and coronary artery disease risk because a significantly positive association between IL-6 levels and CHD risk was found when circulating IL-6 levels were directly used as the exposure variable and treated as a continuous variable.36

### Table 3. Parameter Estimates for the Bivariate AE Models for the Relation of Systemic Inflammation With Coronary Heart Disease Death

<table>
<thead>
<tr>
<th>Model Fitting</th>
<th>Phenotypic Correlation (95% CI)</th>
<th>Components of Phenotypic Correlation†</th>
<th>Genetic Contribution (Actual Value [Relative Value])</th>
<th>Unique Environmental Contribution (Actual Value [Relative Value])</th>
<th>r^g^ (95% CI)</th>
<th>r^e^ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>r_g (95% CI)</td>
<td>r_e (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 and CHD death</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model</td>
<td>0.16 (0.03–0.28)</td>
<td>0.16 (96%)</td>
<td>0.01 (4%)</td>
<td>0.69 (−0.03 to 1.00)</td>
<td>0.01 (−0.22 to 0.24)</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted model</td>
<td>0.26 (0.07–0.42)</td>
<td>0.29 (112%)</td>
<td>−0.03 (−12%)</td>
<td>0.73 (0.18–1.00)</td>
<td>−0.06 (−0.35 to 0.25)</td>
<td></td>
</tr>
<tr>
<td>Score-adjusted model§</td>
<td>0.27 (0.08–0.43)</td>
<td>0.30 (111%)</td>
<td>−0.03 (−11%)</td>
<td>0.74 (0.21–1.00)</td>
<td>−0.05 (−0.35 to 0.25)</td>
<td></td>
</tr>
<tr>
<td>CRP and CHD death</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model</td>
<td>0.11 (−0.02 to 0.23)</td>
<td>0.16 (149%)</td>
<td>−0.05 (−49%)</td>
<td>0.64 (−0.06 to 1.00)</td>
<td>−0.07 (−0.30 to 0.18)</td>
<td></td>
</tr>
<tr>
<td>Age-adjusted model</td>
<td>0.10 (−0.03 to 0.22)</td>
<td>0.15 (155%)</td>
<td>−0.05 (−55%)</td>
<td>0.62 (−0.07 to 1.00)</td>
<td>−0.07 (−0.30 to 0.17)</td>
<td></td>
</tr>
<tr>
<td>Score-adjusted model§</td>
<td>0.10 (−0.02 to 0.22)</td>
<td>0.20 (149%)</td>
<td>−0.09 (−49%)</td>
<td>0.63 (−0.07 to 1.00)</td>
<td>−0.07 (−0.29 to 0.17)</td>
<td></td>
</tr>
</tbody>
</table>

CHD indicates coronary heart disease; CI, confidence interval; CRP, C-reactive protein; and IL-6, interleukin-6.

*A and E refer to additive genetic and unique environmental influences, respectively.

†The phenotypic correlation equals the sum of absolute contribution of genetic and environmental components. Relative contribution of individual component was the percentage of absolute contribution value of each component out of the phenotypic correlation.

‡Adjustment for risk score that contained information about age, smoking, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, diabetes mellitus, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.
and coronary artery disease could not be excluded. Such natural limitations could be avoided in our twin study.

In our twin study, genetic influences were statistically derived using the classic twin design. We found that substantial shared genetic materials contributed to the correlation between IL-6 levels and CHD death. This finding was the genetic evidence to support the causal relation between IL-6 levels and CHD. Mendelian randomization studies are aimed neither to provide genetic evidence for the causal link nor to quantify shared genetic factors. The presence of shared genetic determinants suggested the violation of the soundness of Mendelian randomization. Didelez and Sheehan indicated that “if we know of a gene closely linked to the phenotype without direct effect on the disease, it can often be reasonably assumed that the gene is not itself associated with any confounding factors—a phenomenon called Mendelian randomization.” Our twin study was not restricted to this prerequisite for the Mendelian randomization studies. Findings from Mendelian randomization studies would not be applied to the identification of the high-risk population via genetic variants, whereas those from our twin study should encourage the genetic identification of the high-risk population.

Another Mendelian randomization meta-analysis of pooled 25,458 CHD cases and 100,740 controls from 40 studies attempted to address the causal link between the IL-6 receptor pathway and CHD. Although the T relative to C polymorphism of IL-6 receptor rs7529229 in the IL-6R gene was related to higher circulating concentrations of IL-6 and soluble IL-6 receptor but lower CRP levels, it was not clearly defined whether the genetic variant was used as the proxy for circulating IL-6 levels alone, circulating soluble IL-6 receptor levels alone, circulating CRP levels alone, or for all 3 of circulating inflammatory biomarkers. As IL-6, soluble IL-6 receptor, and CRP levels were involved in the same pathway, the bias from potential genetic pleiotropy could not be excluded. This study reported that the T relative to C polymorphism of IL-6 receptor rs7529229 was associated with decreased odds of CHD events (including deaths). It was concluded that IL-6R signaling seemed to have a causal role in development of CHD. However, the ambiguous definition for the exposure variable raised a concern about interpretations of the study results, suggesting another possible limitation of Mendelian randomization studies.

Similarly, circulating CRP levels in relation to the CHD risk were also investigated in Mendelian randomization studies. Two large-scale Mendelian randomization meta-analyses failed to find statistically significant association of CRP gene variants in the CRP locus with CHD, although there was a significant association between CRP variants and circulating CRP concentrations. The authors did not exclude the causal association because of aforementioned natural limitations of Mendelian randomization studies.

There were limitations in our twin study. Our twins were white men, thus our results may not be generalized to women and other ethnic groups. We could not completely rule out the possibility of residual confounding, although several inflammation-related or CHD-related variables were adjusted for by using a risk score. The assumptions of classical twin studies in general also applied. In particular, common environmental influences were confounded with genetic nonadditivity, so it was possible that common environmental effects were underestimated. The correlation between environmental factors that influenced the traits was assumed to be equal for MZ and DZ twins. This assumption only applied to environmental factors that were not selected or elicited by the twins (eg, choice of diet as opposed to diet selected for them by their parents). It seems unlikely in the case of diet that parents of MZ twins would have systematically given more similar diets than parents of DZ twins. Because CHD was typically experienced later in life, during which time most twin pairs did not cohabit, it was likely that the shared environments of MZ pairs were not systematically more correlated than those of DZ pairs. The average blood sample storage period was 28 years in our study. Because of the limited samples, we did not evaluate the stability of plasma IL-6 and CRP. However, previous studies demonstrated the long-term stability of serum IL-6 and CRP. In our bivariate analysis, we used the CHD death but not time-to-CHD death. To our best knowledge, there was not any established method for bivariate analysis in which 21 variable was time-to-event. Our finding still sheds light on the genetic and environmental contribution to the correlation of inflammation with CHD death risk.

Compared with Mendelian randomization studies and other traditional observational epidemiological studies, our twin study was advantageous in the direct evaluation of the phenotypic correlation between inflammatory biomarkers and CHD and understanding of genetic or environmental components underlying the correlation.

Our findings have clinical and preventive applications. Our results suggest that the therapeutic or interventional lowering of plasma levels of IL-6, either genetically or by medication, would be likely to reduce CHD death. Our findings encourage the identification of specific genetic variants that increased plasma IL-6 levels, which in turn would help clinicians to identify high-risk people for CHD death.

In conclusion, the positive phenotypic correlation between IL-6 and CHD death is driven by genetic factors but attenuated by unique environmental factors. Genetic factors that lead to high plasma levels of IL-6 also lead to CHD death. We could not exclude the possibility that IL-6 and CHD death share unique environmental factors.

Acknowledgments


Sources of Funding

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Disclosures

None.
Significance

To our knowledge, this is the first study to illustrate that low-grade inflammation measured by interleukin-6 and coronary heart death share genetic and unique environmental factors. Plasma interleukin-6 levels may be valuable for coronary heart disease mortality risk stratification and management. Our findings encourage the identification of specific genes that influence both plasma interleukin-6 levels and coronary heart death.
Genetic and Environmental Influences on the Prospective Correlation Between Systemic Inflammation and Coronary Heart Disease Death in Male Twins
Sheng-Hui Wu, Michael C. Neale, Anthony J. Acton, Jr, Robert V. Considine, Ruth E. Krasnow, Terry Reed and Jun Dai

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Material and methods

Study population
The National Heart, Lung, and Blood Institute (NHLBI) Twin Study has been widely described\(^1\)\(^-\)\(^3\). The NHLBI Twin Study was designed to prospectively investigate the genetic and environmental role in cardiovascular risk through inclusion of 514 middle-aged, white male, veteran twin pairs born between 1917 and 1927 at baseline (1969-1973)\(^2\)\(^,\)\(^3\). Based on zygosity ascertained by 8 red blood cell antigen groups (serotyping 22 erythrocyte antigens) in the 1960s and variable number of tandem repeat DNA markers in the 1980s\(^3\), the baseline parent cohort included 253 MZ and 261 DZ pairs\(^3\).

In this study, twins were excluded if they had baseline CHD, plasma levels of IL-6 \(\geq 10\) pg/mL or CRP \(\geq 30\) mg/L\(^4\)\(^,\)\(^5\). A total of 61 twins had baseline CHD diagnosed by the physicians out of the 1028 twins; an additional 17 twins had either IL-6 \(\geq 10\) pg/mL or CRP \(\geq 30\) mg/L out of the 967 remaining twins. In total, 950 twins were included in this study. The reported study was approved by the Institutional Review Boards of Vanderbilt University.

Measures of plasma IL-6 and CRP levels
Blood was drawn from the forearm vein after an overnight fast into EDTA tubes and immediately placed on ice. After centrifugation, plasma aliquots were frozen at -70 °C\(^6\)\(^,\)\(^7\). Plasma IL-6 and CRP concentrations were measured with ELISA (R&D Systems, Minneapolis, Minnesota) and (Millipore, St. Charles, Missouri), respectively. The inter- and intra-assay variability for all assays was < 10%. Plasma samples from a twin pair were assayed in the same analytical run without knowing zygosity.

Baseline data collection
Baseline data were collected on known CHD risk factors following a protocol identical to the Framingham Heart study protocol\(^1\)\(^,\)\(^2\)\(^,\)\(^8\)\(^,\)\(^9\). Height and weight were measured to calculate body mass index. Smoking status (current, past and never smoking) was collected\(^10\). Systolic and diastolic blood pressures were measured using a standard mercury sphygmomanometer\(^10\). A minimum 9-hour overnight fasting blood was drawn to measure triglycerides, total cholesterol, high density lipid cholesterol, and low density lipid cholesterol using the North American Lipid Research Clinics methodology\(^7\). Diabetes was defined as current use of insulin or oral hypoglycemic agents; or a 1-hour 50-gram post-load glucose level > 250 mg/dL\(^10\). A 12-lead electrocardiogram was recorded. Information on medical history and the current use of medications was collected. Heart disease and other cardiovascular disease were diagnosed by the physician at baseline\(^7\).

Assessment of endpoints
We previously described the assessment of endpoints\(^2\). Vital status as well as the cause and date of death through December 31, 2010 were ascertained from medical records and death certificates in four follow-up examinations (exam 2, 1981-1982; exam 3, 1986-1987; exam 4, 1995-1997; and exam 5, 1999-2000), and through death certificates or the National Death Index after exam 5\(^7\). Criteria used for ascertaining outcomes in four follow-up examinations were standardized, and decisions regarding disease diagnosis were made by a panel of investigators: at examinations 2 and 3 two independent physicians reviewed medical records; at examinations 4 and 5 one physician reviewed medical records. Physicians assigned corresponding International Classification of Diseases Ninth Revision codes. Death certificates or the National Death Index with the Ninth Revision codes were obtained for decedents. The primary endpoint was death from coronary heart disease (410-414). Secondary endpoints were death from cardiovascular diseases (390-398, 402, 404, 410-438) and all causes. Subjects were considered lost to follow-up if a death certificate or coding from the National Death Index could not be traced.

**Statistical analysis**

Plasma levels of IL-6 and CRP concentrations were natural log-transformed and treated as continuous variables for analyses.

*Composite risk score*

We constructed a composite risk score calculated as the logit of a propensity score \(p\), i.e. \(\log\left(\frac{p}{1-p}\right)\) following the published method\(^11\). Using generalized estimating equation logistic analysis, we obtained the propensity score as the probability of twins to die of CHD. In the generalized estimating equation model, independent variables were 10 known CHD risk factors at baseline, including age, smoking, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, diabetes, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol. The risk score was used to avoid potential model over-fitting and to reduce the bias due to imbalance of known CHD risk factors between twins who died of CHD and those who did not die of CHD in subsequent structural equation modeling analyses.

*Univariate analyses*

We estimated genetic and environmental influences on the measured traits (i.e. IL-6, CRP, and CHD death) using structural equation modeling, specifically A (additive genetics) C (common environment) E (unique environment) and AD(non-additive genetics)E models\(^12,\,13\). In this modeling, genetic factors were conceptualized as genetic components (A and D), and environmental factors were conceptualized as environmental components (C and E). These components were not physically measured in the study and thus called “latent” variables. The A comprised the sum of average effects of the individual alleles at different loci. The D was the interactions between alleles at the same locus,
or from interaction of genes at different loci. The C was trait-influencing environmental factors shared between co-twins within a twin pair; they increase co-twin similarity. The E referred to environmental factors that were not shared between co-twins within a twin pair and made co-twins different from each other. The quantification of these latent variables, ACDE, was based on comparisons of the similarity between MZ and DZ twins on a measured trait like IL-6. MZ twins share 100% of their genes, while DZ twins share on average 50% of their genes. For a genetically influenced trait, MZ twins would be more similar than DZ twins regarding the trait. Both ACE and ADE models had several nested reduced models: AE, CE and E for ACE; and AE, DE and E for ADE. The best model was selected based on the goodness of fit to data and the model parsimony. A smaller Akaike’s Information Criterion indicated a better fit to the data.

**Bivariate analyses**

In bivariate analyses, we tested whether inflammation was correlated with CHD death, and whether genetic and environmental factors that influenced inflammation also affected CHD death. Bivariate Cholesky decomposition models were fitted to the covariance matrices by the maximum likelihood method. Because the AE model showed the best fit in the univariate and bivariate genetic analyses, we used this model in all bivariate analyses.

**Supplemental Figure I** shows a bivariate Cholesky AE model, where A was additive genetic influences on the phenotypic correlation of one biomarker with CHD death while E was unique environmental influence on the correlation. The phenotypic correlation was conceptually caused by mixed contributions from latent genetic and unique environmental components. Mathematically, the phenotypic correlation coefficient was the sum of the estimated contributions of both genetic and environmental components. The phenotypic correlation between one biomarker and CHD death was partitioned into two components: one due to genetic components \((a_{11} \times a_{21})/\sqrt{(a_{11}^2 + e_{11}^2) \times (a_{22}^2 + e_{22}^2)}\), and the other one due to unique environmental factors \((e_{11} \times e_{21})/\sqrt{(a_{11}^2 + e_{11}^2) \times (a_{22}^2 + e_{22}^2)}\). To elucidate the relative effect of each component on the correlation, the relative contributions of each component were also calculated as the proportion of the estimated contribution of each component out of the phenotypic correlation coefficient. The relative contribution of genetic components to the phenotypic correlation was calculated as \(a_{11} \times a_{21}/(a_{11} \times a_{21} + e_{11} \times e_{21})\), and the relative contribution of unique environment was equal to \(e_{11} \times e_{21}/(a_{11} \times a_{21} + e_{11} \times e_{21})\). In our best fitting AE model, the sum of relative genetic and unique environmental contributions was set to 1. The relative contribution of both genetic and unique environmental factors could range from 0 to 1. It is possible that the relative genetic contribution exceeds 1 and the relative unique environmental
is negative, or vice versa, which would occur if the genetic and unique environmental factors contributed to the phenotypic correlation in opposite directions. These contributions denote genetic and environmental contributions to the phenotypic correlation of one biomarker with CHD death\textsuperscript{12}. If the relative contribution of one component was less than 0, the relative contribution of the other would exceed 1. This scenario would occur if A and E contributed to the phenotypic correlation in opposite directions.

The genetic and unique environmental factors contributing to the phenotypic correlation could be shared between the two traits completely, partially, or not at all. The extent that genes were overlapped between the two traits was further evaluated through the genetic correlation coefficient \( r_g \). An \( r_g \) of 1.0 indicates that genetic influences on the two traits overlap completely, i.e., the same set of genetic factors influenced both inflammation and CHD death. An \( r_g \) of 0 indicated that the effects of genes influencing one trait were independent of those on the other trait\textsuperscript{14}, i.e., genetic factors influencing on inflammation were completely different from those on CHD death. Similarly, the unique environmental correlation coefficient \( r_e \) estimates the degree to which unique environmental factors involved in the phenotypic correlation were shared between two traits\textsuperscript{12}. An \( r_e \) of 1.0 indicates that unique environmental influences on both traits overlap completely, whereas an \( r_e \) of 0 would indicate that the effects of unique environmental factors on one trait are independent of those on the second. An \( r_e \) less than 0 indicates that unique environmental factors influencing the two traits do so in opposite directions. A correlation coefficient confidence interval that includes 0 indicates a statistically nonsignificant correlation; otherwise, the confidence interval indicates a statistically significant correlation\textsuperscript{12}.

Furthermore, we calculated the proportion of the heritability for CHD death due to genetic factors shared between CHD death and inflammation as the absolute value of \( r_g \) (\(|r_g|\)) and the proportion due to genetic factors independent of inflammation as \((1-|r_g|)^{15}\). This approach allowed us to address questions: of the genetic factors influencing CHD death, what proportion was specific to CHD death, and what proportion was due to factors that also influenced inflammation. The similar calculation was also conducted for unique environmental origin of the CHD death using \( r_e \).

In the univariate and bivariate analyses, we first adjusted for age at baseline. Then, we adjusted for the risk score that included information on age and other traditional CHD risk factors as described above.

In the sensitivity analysis, we examined the results with additional adjustment for plasma sample collection examination. In another sensitivity analysis, we examined the robustness of our findings by the exclusion of twins with missing
biomarker data.

Structural equation modeling analysis was conducted using OpenMx\textsuperscript{16}. The other analyses were performed using SAS 9.1 statistical package (SAS Institute, Cary, NC).

References


Supplemental Material

Supplemental Table I. Multivariable adjusted parameter estimates for the bivariate AE models of systemic inflammation with coronary heart disease death

<table>
<thead>
<tr>
<th>Model fitting</th>
<th>Phenotypic correlation (95% CI)</th>
<th>Components of phenotypic correlation*</th>
<th>( r_g ) (95% CI)</th>
<th>( r_e ) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Genetic contribution [actual value (relative value)]</td>
<td>Unique environmental contribution [actual value (relative value)]</td>
<td></td>
</tr>
<tr>
<td>IL-6 and CHD death</td>
<td>0.27 (0.08, 0.43)</td>
<td>0.30 (111%)</td>
<td>-0.03 (-11%)</td>
<td>0.74 (0.21, 1.00)</td>
</tr>
<tr>
<td>Multivariable adjusted model( ^$ )</td>
<td>1.01 (0.92, 1.11)</td>
<td>0.30 (111%)</td>
<td>-0.03 (-11%)</td>
<td>0.74 (0.21, 1.00)</td>
</tr>
<tr>
<td>CRP and CHD death</td>
<td>0.10 (-0.02, 0.22)</td>
<td>0.15 (149%)</td>
<td>-0.05 (-49%)</td>
<td>0.63 (-0.07, 1.00)</td>
</tr>
</tbody>
</table>

CI: confidence interval; CRP: C-reactive protein; IL-6: interleukin-6.
A and E refer to additive genetic and unique environmental influences, respectively.
\( ^* \) \( r_g \) and \( r_e \) represented genetic and environmental correlation\( ^1 \).
\( ^\$ \) The phenotypic correlation equals the sum of absolute contribution of genetic and environmental components. Relative contribution of individual component was the percentage of absolute contribution value of each component out of the phenotypic correlation.
\( ^\$ \) Adjustment for risk score and plasma sample collection examinations. The risk score contained information about age, smoking, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, diabetes, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.
Supplemental Table II. Parameter estimates for the bivariate AE models for the relation of systemic inflammation with cardiovascular disease (CVD) and all-cause mortality

<table>
<thead>
<tr>
<th>Model fitting</th>
<th>Phenotypic correlation (95% CI)</th>
<th>Components of phenotypic correlation</th>
<th>r_g (95% CI)</th>
<th>r_e (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CVD Death (n=241)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IL-6 and CVD mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model</td>
<td>0.17 (0.06, 0.28)</td>
<td>0.19 (109%)</td>
<td>0.82 (0.20, 1.00)</td>
<td>-0.02 (-0.21, 0.18)</td>
</tr>
<tr>
<td>Age-adjusted model</td>
<td>0.26 (0.09, 0.40)</td>
<td>0.30 (117%)</td>
<td>0.77 (0.28, 1.00)</td>
<td>-0.08 (-0.35, 0.19)</td>
</tr>
<tr>
<td>Score-adjusted model</td>
<td>0.27 (0.11, 0.41)</td>
<td>0.32 (117%)</td>
<td>0.79 (0.33, 1.00)</td>
<td>-0.08 (-0.35, 0.19)</td>
</tr>
<tr>
<td><strong>CRP and CVD mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model</td>
<td>0.14 (0.03, 0.25)</td>
<td>0.19 (136%)</td>
<td>0.72 (0.18, 1.00)</td>
<td>-0.07 (-0.27, 0.14)</td>
</tr>
<tr>
<td>Age-adjusted model</td>
<td>0.13 (0.02, 0.23)</td>
<td>0.18 (138%)</td>
<td>0.71 (0.15, 1.00)</td>
<td>-0.06 (-0.26, 0.14)</td>
</tr>
<tr>
<td>Score-adjusted model</td>
<td>0.13 (0.03, 0.21)</td>
<td>0.18 (135%)</td>
<td>0.72 (0.17, 1.00)</td>
<td>-0.06 (-0.26, 0.14)</td>
</tr>
<tr>
<td>All-cause Death (n=688)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IL-6 and all-cause mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model</td>
<td>0.30 (0.19, 0.40)</td>
<td>0.23 (76%)</td>
<td>0.76 (0.33, 1.00)</td>
<td>0.10 (-0.12, 0.32)</td>
</tr>
<tr>
<td>Age-adjusted model</td>
<td>0.42 (0.27, 0.54)</td>
<td>0.35 (84%)</td>
<td>0.69 (0.38, 0.98)</td>
<td>0.14 (-0.14, 0.40)</td>
</tr>
<tr>
<td>Score-adjusted model</td>
<td>0.43 (0.29, 0.55)</td>
<td>0.37 (85%)</td>
<td>0.70 (0.41, 0.98)</td>
<td>0.14 (-0.14, 0.41)</td>
</tr>
<tr>
<td><strong>CRP and all-cause mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude model</td>
<td>0.15 (0.03, 0.25)</td>
<td>0.23 (151%)</td>
<td>0.65 (0.25, 1.00)</td>
<td>-0.11 (-0.33, 0.11)</td>
</tr>
<tr>
<td>Age-adjusted model</td>
<td>0.13 (0.02, 0.24)</td>
<td>0.20 (155%)</td>
<td>0.63 (0.23, 1.00)</td>
<td>-0.11 (-0.32, 0.11)</td>
</tr>
<tr>
<td>Score-adjusted model</td>
<td>0.14 (0.03, 0.24)</td>
<td>0.21 (152%)</td>
<td>0.65 (0.25, 1.00)</td>
<td>-0.11 (-0.32, 0.11)</td>
</tr>
</tbody>
</table>

CI: confidence interval; CRP: C-reactive protein; IL-6: interleukin-6.

1 A and E refer to additive genetic and unique environmental influences, respectively.

2 r_g and r_e represented genetic and environmental correlation.

3 The phenotypic correlation equals the sum of absolute contribution of genetic and environmental components. Relative contribution of individual component was the percentage of absolute contribution value of each component out of the phenotypic correlation.
§Adjustment for risk score that contained information about age, smoking, body mass index, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, diabetes, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol.
Twin 1
IL-6 or CRP

A_1
E_1

1

1/0.5

r_g

1/0.5

r_e

Twin 1
CHD

A_2
E_2

1

1

a_11

e_11

a_21

e_21

a_22

e_22

Twin 2
IL-6 or CRP

A_1
E_1

1

1/0.5

r_g

1/0.5

r_e

Twin 2
CHD

A_2
E_2

1

1

a_11

e_11

a_21

e_21

a_22

e_22

1/0.5
Supplemental Figure I. Cholesky decomposition of correlation of interleukin-6 (IL-6) or C-reactive protein (CRP) with coronary heart disease (CHD) death. Latent variables are represented by circles and phenotypes are represented by boxes (AE model). A and E are genetic and unique environmental influences to IL-6 or CRP and CHD death; the effects of A and E are represented by parameters a and e respectively. $r_g$ and $r_e$ represented genetic and environmental correlation of IL-6 and CRP with CHD death respectively.
Reference