Coronary Artery Calcification Progression Is Heritable

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Background—Coronary artery calcification (CAC), a marker of coronary artery atherosclerosis, can be measured accurately and noninvasively with the use of electron beam computed tomography. Serial measures of CAC quantify progression of calcified coronary artery plaque. Little is known about the role of genetic factors in progression of CAC quantity.

Methods and Results—We quantified the relative contributions of measured risk factors and unmeasured genes to CAC progression measured by 2 electron beam computed tomography examinations an average of 7.3 years apart in 877 asymptomatic white adults (46% men) from 625 families in a community-based sample. After adjustment for baseline risk factors and CAC quantity, the estimated heritability of CAC progression was 0.40 (P<0.001). Baseline risk factors and CAC quantity explained 64% of the variation in CAC progression. Thus, genetic factors explained 14% of the variation [(100−64)×(0.40)] in CAC progression. After adjustment for risk factors, the estimated genetic correlation (pleiotropy) between baseline CAC quantity and CAC progression was 0.80 and was significantly different than 0 (P<0.001) and 1 (P=0.037). The environmental correlation between baseline CAC quantity and CAC progression was 0.42 and was significantly different than 0 (P=0.006).

Conclusions—Evidence was found that many but not all genetic factors influencing baseline CAC quantity also influence CAC progression. The identification of common and unique genetic influences on these traits will provide important insights into the genetic architecture of coronary artery atherosclerosis. (Circulation. 2007;116:25-31.)

Key Words: atherosclerosis ■ calcium ■ genetics ■ imaging ■ population

Coronary Heart Disease

Coronary artery calcification (CAC), a marker of coronary atherosclerosis, can be measured accurately and noninvasively with electron beam computed tomography. CAC progression can be used to serially measure the progression of calcified coronary artery plaque. Little is known about the role of genetic factors in CAC progression.

Methods

We quantified the relative contributions of measured risk factors and unmeasured genes to CAC progression measured by 2 electron beam computed tomography examinations an average of 7.3 years apart in 877 asymptomatic white adults (46% men) from 625 families in a community-based sample. After adjustment for baseline risk factors and CAC quantity, the estimated heritability of CAC progression was 0.40 (P<0.001). Baseline risk factors and CAC quantity explained 64% of the variation in CAC progression. Thus, genetic factors explained 14% of the variation [(100−64)×(0.40)] in CAC progression. After adjustment for risk factors, the estimated genetic correlation (pleiotropy) between baseline CAC quantity and CAC progression was 0.80 and was significantly different than 0 (P<0.001) and 1 (P=0.037). The environmental correlation between baseline CAC quantity and CAC progression was 0.42 and was significantly different than 0 (P=0.006).

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Evidence was found that many but not all genetic factors influencing baseline CAC quantity also influence CAC progression. The identification of common and unique genetic influences on these traits will provide important insights into the genetic architecture of coronary artery atherosclerosis.

Key Words: atherosclerosis ■ calcium ■ genetics ■ imaging ■ population

Clinical Perspective p 31

Atherosclerosis is the primary cause of CHD. Coronary artery calcification (CAC), a measure of coronary atherosclerosis presence and quantity, can be detected noninvasively and reliably with electron beam computed tomography (EBCT). CAC predicts CHD events in asymptomatic individuals at intermediate risk on the basis of their CHD risk factors.3,4 EBCT can be used to serially measure the progression of CAC. CAC progression is associated with CHD.5,6 Family history of premature CHD is associated with CAC.7 Unmeasured genes contribute to interindividual variation in CAC quantity measured at a single time point across studies. Estimated heritability (±SE) was 0.42±0.13 among asymptomatic white individuals,8 0.40±0.08 among siblings enhanced for hypertension,9 and 0.40±0.23 among individuals from families enriched for type 2 diabetes.10

No studies have focused on estimating the genetic contribution to CAC progression, although the complex biology of progression of calcium appears to be “genetically directed.”11 The purpose of the present investigation was to estimate the genetic contribution to variation in noninvasively measured CAC progression among an asymptomatic community-based sample. Additionally, evidence for pleiotropy, or shared genetic influences, between CAC quantity at baseline and CAC progression was examined.

Methods

Study Participants

The Epidemiology of Coronary Artery Calcification (ECAC) study, conducted between 1991 and 1998, examined 1240 participants aged ≥20 years from the Rochester Family Heart Study12,13 and 496 individuals living in the vicinity of Rochester, Minn, who were not...
pregnant or lactating and who never had coronary or noncoronary heart surgery. A total of 1155 ECAC study participants had a follow-up examination between December 2000 and February 2005. In general, participants were invited to return for a follow-up examination on the basis of age (older age first) and longer time since baseline examination. Study protocols were approved by the Mayo Clinic and University of Michigan institutional review boards, and participants gave written informed consent.

One thousand fifty-five white ECAC participants had complete CAC data at baseline and follow-up and no history of myocardial infarction, stroke, or positive angiogram at baseline or follow-up. Mayo Clinic and University of Michigan institutional review boards, since baseline examination. Study protocols were approved by the

In general, participants were invited to return for a follow-up examination on the basis of age (older age first) and longer time since baseline examination. Study protocols were approved by the Mayo Clinic and University of Michigan institutional review boards, and participants gave written informed consent.

Measurement of CAC
CAC was measured with an Imatron C-150 EBCt scanner (Imatron Inc., South San Francisco, Calif). Protocols at baseline and follow-up were identical. A dual-scan approach was used beginning in 1993. A scan run consisted of 40 contiguous 3-mm-thick tomographic slices from the root of the aorta to the apex of the heart. Scan time was 100 ms per tomogram. ECG gating was used, and all images were triggered at end-diastole during 2 to 4 breath-holds. A radiological technologist scored the tomograms with an automated scoring system without knowledge of other EBCT examination results for the same participant. CAC was defined as a hyperattenuating focus within 5 mm of the midline of a coronary artery, ≥4 contiguous pixels in size, and having CT numbers >130 Hounsfield units throughout. Areas ≥1 mm² for all CAC foci were summed to provide a measure of CAC quantity. When 2 scan runs at a single examination were available, CAC quantity was based on the average.

Statistical Analysis
Baseline CAC quantity was natural logarithm (log) transformed after adding 1 to reduce nonnormality and is referred to as log baseline CAC quantity. CAC progression was defined as the log annual change in CAC area, calculated as follows: log [(difference between follow-up and baseline CAC area+1)/time (in years) between baseline and follow-up examinations]. If the difference between follow-up and baseline CAC area was <0, the difference was set to 0 (to avoid taking the log of a negative number).

Heritability estimates ($h^2$) were calculated for log baseline CAC quantity and CAC progression with the use of a variance component approach described previously and implemented in SOLAR. For trait y, the value of y for individual i is modeled as:

$$y_i = \mu + \sum \beta X_{ij} + g_i + e_i$$

where $\mu$ is the mean of y, $X_{ij}$ is the j-th covariate with associated regression coefficient $\beta$, $g_i$ is an additive genetic effect normally distributed with mean 0 and variance $\sigma^2_g$, and $e_i$ is a residual effect normally distributed with mean 0 and variance $\sigma^2_e$. It is assumed that $\sigma^2_g + \sigma^2_e = 1$. Any nonadditive genetic and unmeasured nongenetic effects (as well as measurement and random error) are incorporated into $e_i$. Heritability is estimated by $\sigma^2_g$. Likelihood ratio tests are used to assess significance of a parameter of interest by comparing the log-likelihood of the model in which the parameter is estimated with that of the model in which the parameter is fixed to 0.

Heritability estimates for CAC progression were calculated as follows: (1) unadjusted; (2) adjusted for age and sex; (3) adjusted for age, sex, and the best subset of the following baseline CHD risk factors: body mass index, waist-to-hip ratio, triglycerides, LDL-C, HDL-C, fasting glucose level, SBP, DBP, presence of diabetes, presence of hypertension, college education (ie, any education beyond high school), smoking history, log (pack-years smoking+1), and family history of CHD; and (4) adjusted for age, sex, log baseline CAC quantity, and the best subset of the CHD risk factors listed in step 3. Heritability estimates for log baseline CAC quantity were calculated similarly (steps 1 to 3). Covariates were chosen for similarity to previous h² studies. All 2-way interaction terms between covariates significantly associated with either outcome were evaluated. The estimates of h² and covariate variance obtained were used to estimate the percentage of total variation explained by genetic factors: [(1−proportion of variation explained by covariates)×h²]×100.

The genetic correlation ($\Psi$) between log baseline CAC quantity (trait 1) and CAC progression (trait 2) was estimated to assess pleiotropic genetic effects with the use of maximum-likelihood estimation in SOLAR. The phenotypic correlation between the 2 traits is derived from the $\Psi$, the environmental correlation ($\Psi_e$), and the heritabilities of the 2 traits, as follows:

$$\Psi = \frac{\text{cov}(X_1, X_2)}{\sqrt{\text{var}(X_1)\times\text{var}(X_2)}}$$

All hypothesis tests were performed with the use of likelihood-ratio test statistics. The hypothesis tests of interest are whether $\Psi$ is different from 0, whether $\Psi_e$ is different from 1, and whether $\Psi$ is different from 0. If $\Psi$, is different from 0, the estimate of $\Psi_e$, its SE, and test of the hypothesis $\Psi_e=1$ determine the magnitude of the shared genetic effects (ie, pleiotropy). If the hypothesis that $\Psi=1$ is not rejected, then all genes influencing 1 trait are assumed to also influence the other trait. Rejection of the null hypothesis that $\Psi=0$ indicates shared environmental components. Covariates significantly associated with both traits were used to adjust both traits, whereas covariates only associated with a single trait were used to adjust for that trait alone. Covariates for CAC progression were chosen from the model in which log baseline CAC quantity was not included as a covariate.

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**
Mean baseline age of women was 56.4 years (range, 36.0 to 82.1 years), and that of men was 54.7 years (range, 35.7 to 79.0 years) (Table 1). Mean time between examinations was...
TABLE 2. Distribution of CAC Quantity at Baseline and Follow-Up and CAC Progression, by Sex

<table>
<thead>
<tr>
<th>CAC Measure Baseline Follow-Up Annual Change per Year*</th>
<th>Women</th>
<th>Men</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAC quantity, mm²</td>
<td>21.7 (9.7) [0, 56.7]</td>
<td>41.3 (11.6) [0, 110.7]</td>
<td>3.7 (0.6) [−3.1, 10.0]</td>
</tr>
<tr>
<td>Log [CAC quantity + 1]</td>
<td>1.1 (0.7) [0, 6.9]</td>
<td>1.8 (0.9) [0, 7.0]</td>
<td>−0.4 (0.8) [−2.6, 4.6]</td>
</tr>
<tr>
<td>Presence of any detectable CAC, %</td>
<td>38.1</td>
<td>58.0</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data are mean (SD) unless indicated otherwise.

*On scale of mm²/y, defined as (follow-up—baseline CAC quantity/time) (mm²/y); on log scale, defined as CAC progression: [|log((follow-up—baseline CAC quantity + 1)/time)].
TABLE 3. Baseline Risk Factors Associated With Log Baseline CAC Quantity and/or With CAC Progression

<table>
<thead>
<tr>
<th>Baseline Covariate</th>
<th>Log Baseline CAC Quantity</th>
<th>CAC Progression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parameter Estimate (SE)</td>
<td>P</td>
</tr>
<tr>
<td>Age</td>
<td>0.075 (0.006)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female sex</td>
<td>-1.115 (0.102)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>0.145 (0.087)</td>
<td>0.107</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>0.023 (0.004)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>-0.016 (0.007)</td>
<td>0.016</td>
</tr>
<tr>
<td>Log (pack-years of smoking + 1)</td>
<td>0.202 (0.033)</td>
<td>0.002</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.984 (0.345)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Family history of CHD</td>
<td>0.262 (0.109)</td>
<td>0.029</td>
</tr>
<tr>
<td>Sex×LDL-C</td>
<td>-0.255 (0.120)</td>
<td>0.020</td>
</tr>
<tr>
<td>Log baseline CAC quantity</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Age×log baseline CAC quantity</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Ellipses refer to variable not selected in stepwise regression procedure in SOLAR. NA indicates not applicable.

Discussion

The present study is the first to estimate the genetic contribution to CAC progression. There is evidence to suggest a strong, shared genetic component to both CAC quantity at a single time point and CAC progression, but there is also evidence suggesting that unique genes are involved in each of these measures of subclinical coronary artery atherosclerosis. Although no one has identified candidate genes associated with the rate of progression of CAC, others have identified candidate genes associated with CAC progression when defined as a qualitative trait (ie, progressors versus nonprogressors\(^30-31\)) in individuals with type 1 diabetes.\(^30-31\) It would be important to investigate whether any identified genes are unique for CAC progression or whether they also are associated with cross-sectional measures of CAC prevalence or quantity.

Several clinical trials\(^32-34\) examining LDL-C reduction through statin therapy and CAC progression have recently been published. These studies evaluated change in CAC over a short period of time (\(\leq 3\) years) in study populations with specific characteristics (hyperlipidemic and postmenopausal women\(^32\); patients with \(\geq 2\) CAD risk factors plus moderate calcification\(^33\); patients with calcific aortic stenosis\(^34\)). Despite a reduction in LDL-C, there was no evidence of a slowing of CAC progression. In the present study, however, baseline LDL-C was positively associated with increased CAC progression over a much longer follow-up period in a community-based sample. This suggests that LDL-C levels may be early in the development and progression of atherosclerosis; our finding is consistent with that of Kuller et al\(^35\) (1999), who showed that premenopausal LDL-C levels were powerful predictors of CAC measured 8 years after menopause (11 years after LDL-C measurement). Future work examining the effect of LDL-C reduction on CAC progression over an extended follow-up period may be warranted. Additionally, studies examining LDL-C reduction in preventing detectable CAC development among those without detectable CAC may reveal additional insight into the pathogenesis of LDL-C-mediated CAC development and/or progression. It may also be of use to examine age- and sex-specific effects of LDL-C reduction on CAC progression.

Limitations

Approximately one half of individuals did not belong to a sibship. Although these individuals contributed information to estimation of the mean and variance of the traits being investigated, as well as to relationships between covariates and traits of interest, they did not contribute information to the heritability estimation. However, our baseline \(h^2\) estimates and their SEs closely resemble those obtained by others,\(^8-10\) suggesting that our sample is sufficient for estimating \(h^2\) of CAC progression.

In the present study, \(h^2\) estimates may overestimate the genetic contribution because we have not estimated shared environments. All siblings reported living in separate households from one another and their parents at the time of the study. However, shared environments early in life may contribute to
the correlations for CAC quantity and CAC progression seen among adult relatives.

Our study sample was restricted to white individuals; however, CAC burden and progression vary across different ethnic populations. Thus, future studies examining the genetic contribution to CAC progression in other ethnic groups are warranted.

Participants whose follow-up CAC quantity was less than CAC quantity at baseline (n = 52; 5.9%) were treated as having no change in the definition of CAC progression. The mean change in this group was 1.3 mm²/y. Individuals with less detectable CAC at follow-up compared with baseline examination were younger (mean age, 52.8 ± 11.7 versus 55.8 ± 10.1 years; P = 0.042), had larger mean body mass index (30.1 ± 5.3 versus 27.4 ± 4.8 kg/m²; P < 0.001), had larger mean waist-to-hip ratio (0.89 ± 0.09 versus 0.85 ± 0.10; P = 0.018), and were less likely to report a family history of CHD (13.5% versus 35.2%; P = 0.011) than the remainder of the study sample. Only 28 (46.2%) of these 52 participants had any detectable CAC at follow-up examination; these 28 individuals had small quantities of detectable CAC at baseline (mean, 2.7 ± 3.1 mm²; range, 0.7 to 12.2 mm²). The negative differences between baseline and follow-up are likely attributable to measurement errors rather than being true regression of CAC because larger body size creates additional noise in CAC measurement, and 40% of those with less detectable CAC at follow-up compared with baseline had small CAC quantity detected at baseline and no detectable CAC at follow-up. Furthermore, after we repeated our analyses removing these 52 participants from the sample, our inferences remained the same. Thus, treatment of these participants as having no change between baseline and follow-up is reasonable, particularly because evidence from animal studies indicates that although calcium progression itself may be slowed or stopped (eg, through dietary intervention), there is no evidence suggesting that calcium deposits will exhibit a true regression in the absence of aggressive intervention.

Although a direct relationship exists between CAC and both histological and in vivo measures of atherosclerotic plaque on a
heart-by-heart, vessel-by-vessel, and segment-by-segment basis, absence of detectable CAC with EBCT does not necessarily indicate an absence of coronary artery atherosclerosis. This measure likely underestimates total atherosclerosis quantity and progression in some individuals because CAC quantity more closely represents calcified plaque burden rather than atherosclerosis.

Finally, we restricted our analyses to account for baseline measures of risk factors only; however, change in risk factor status over time may retard or accelerate CAC progression with unknown effects on estimation of the role of genetic factors. Future work should examine time-varying covariates in CAC progression.

Conclusion

Both individual and familial characteristics (eg, genes) are important factors in CAC progression. Importantly, there is a genetic component to CAC progression beyond that captured by baseline risk factors (including family history of CHD) and baseline CAC. Baseline risk factors (including family history of CHD) and baseline CAC may provide useful tools for identifying individuals at otherwise low to moderate risk of a CHD event who may benefit from serial CAC screening for additional risk stratification and/or primary prevention of disease.

Identification of specific genes associated with increased CAC progression may provide insights into molecular mechanisms of atherosclerosis, identify new targets for therapy, and lead to blood tests for early detection of susceptible individuals who would benefit from early, individualized therapeutic or lifestyle interventions for halting or slowing their CAC progression.

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Disclosures

None.

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