Genotype at the $-174G/C$ Polymorphism of the Interleukin-6 Gene Is Associated With Common Carotid Artery Intimal-Medial Thickness

Family Study and Meta-Analysis

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Background and Purpose—Studies in unrelated individuals have produced conflicting findings concerning the putative association between the interleukin-6 (IL-6) $-174G/C$ polymorphism and carotid intimal-medial thickness (IMT). We have used a family-based genetic association design to assess the heritability of carotid IMT and to investigate the hypothesized association of carotid IMT with the IL-6 to $-174G/C$ polymorphism.

Methods—We studied 854 members of 224 white British families. The heritability of carotid IMT was determined using a recessive model (GG versus GC+CC). Genomic DNA was extracted from peripheral blood samples and the genotype at the IL-6 to $-174G/C$ polymorphism was determined. We used Multipoint Engine for Rapid Likelihood Inference. Genetic association analyses were carried out using ANOVA and family-based tests of association implemented in Quantitative Transmission Disequilibrium Test. A meta-analysis of previous studies of the association was conducted to place our result in context.

Results—The heritability of carotid IMT was 24%. Under a recessive model (GG+GC versus CC), there was significant evidence of association between IL-6 and carotid IMT (adj$log_{10}$ maximal carotid IMT ($F=5.469$, $P=0.023$). Family-based analyses using Quantitative Transmission Disequilibrium Test showed no evidence of population stratification as a cause of the observed association ($\chi^2=0.469$, $P=0.4934$). The CC genotype was associated with a 4.8% increase in maximal carotid IMT and accounted for 0.6% of the observed variation in the trait, which is equivalent to 2.5% of the heritable component. A meta-analysis of the present and 2 previous large studies, which enrolled a total of 2930 subjects, confirmed the recessive effect of the C allele on carotid IMT ($P=0.0014$).

Conclusions—The genotype at the IL-6 to $-174G/C$ polymorphism is associated with common carotid artery IMT, although the size of the genetic effect is small. (Stroke. 2005;36:2215-2219.)

Key Words: atherosclerosis ■ carotid arteries ■ carotid intimal medial thickness ■ genetics

Atherosclerotic cardiovascular disease aggregates in families in a manner that strongly suggests that genetic factors are important. Carotid artery intimal-medial thickness (IMT) is a subclinical marker of systemic atherosclerosis. There is a direct relationship between carotid IMT and the risk of cardiovascular disease, such that for each 0.03-mm increase per year in carotid IMT, the relative risk for myocardial infarction and stroke is increased 2 to 3. Thus, carotid IMT may be regarded as a quantitative intermediate marker of atherosclerosis that may be suitable for use in genetic studies, should it prove to be heritable. Studies to date have reported widely varying estimates of heritability. Accurate information regarding the heritability of a trait is a prerequisite for the design of adequately powered genetic studies.

Interleukin-6 (IL-6) is a cytokine that plays a key role in driving the acute phase response by orchestrating the production of C-reactive protein and fibrinogen. IL-6 has been associated with several markers of endothelial dysfunction, and, as such, may play a role in the pathogenesis of atherosclerotic vascular disease by direct endothelial activation or indirectly through stimulation of C-reactive protein and fibrinogen synthesis. Several groups have provided evidence indicating that the promoter polymorphism at position $-174$ of the IL-6 gene ($-174G/C$) may itself be a...
functional change that affects the affinity of nuclear proteins involved in the transcription of the gene,\textsuperscript{11,12} raising the possibility that the polymorphism may be a genetic risk factor for vascular inflammation and, thus, atherosclerosis.

Four studies to date have examined the association of the IL-6 to $-174 G/C$ polymorphism and carotid IMT.\textsuperscript{10,13–15} Only 1 of these studies has reported a significant association of the IL-6 to $-174 G/C$ polymorphism and carotid IMT in the whole sample,\textsuperscript{13} whereas the other 3 studies found significant association in smaller subgroup analyses, such as in association with the $MMP3 -16126 A$ polymorphism,\textsuperscript{10} in heavy alcohol users,\textsuperscript{14} and in older subjects.\textsuperscript{15} Such emphasis on retrospectively selected subgroups may yield misleading conclusions.\textsuperscript{16} Additionally, the reported associations in these studies have been in different directions, the smaller studies (including a total of 162 individuals) having found that the $G$ allele is associated with increased carotid IMT,\textsuperscript{10,13} and the larger studies (including a total of 2109 individuals) having found the opposite.\textsuperscript{14,15} Clearly, until additional studies and systematic reviews resolve these discrepancies, the association of the IL-6 genotype with carotid IMT remains unproven.\textsuperscript{17}

We have carried out a family study to determine the heritability of carotid IMT and to investigate the genetic association with the IL-6 to $-174 G/C$ polymorphism in a large panel of extended families that is of proven value in detecting genetic factors influencing quantitative traits.\textsuperscript{18–20}

### Methods

#### Family Collection

Between 1993 and 1997, white British families were ascertained from hypertensive probands for a quantitative genetic study of cardiovascular risk factors.\textsuperscript{18–20} The local ethics committee approved the study; all of the subjects gave written informed consent. There were 248 families with 1425 members collected; the ascertainment strategy has been described previously.\textsuperscript{18–20} A full clinical history, anthropometric measurements, 24-hour ambulatory blood pressure readings, and blood samples were obtained.

#### Genotyping

The IL-6 to $-174G/C$ polymorphism was genotyped as described previously.\textsuperscript{19} Mendelian inheritance of genotypes was verified using PedCheck.\textsuperscript{21}

#### Ultrasound Imaging

Between 1999 and 2001, families were invited to attend for additional phenotyping; at this visit, 12-lead electrocardiograms, echocardiograms, ultrasound measurement of carotid IMT, and 24-hour urine collections for steroid metabolites were performed. Carotid artery ultrasonography was performed by 2 sonographers using a 7.5-MHz linear array transducer, and all of the measurements were made by 1 physician (B.M.M.). The scanning protocol involved studying the right and left common carotid arteries in all of the subjects according to a standard method (see Carotid Intima Media Thickness Measurement section available online only at http://www.strokeaha.org).\textsuperscript{22,23} All of the scans were recorded on an optical disc for off-line analysis. End-diastolic frames (1 from each side of the body) were analyzed for mean and maximal IMT, and the average reading from these 2 frames was calculated. Scans of sufficient quality were analyzed with a computerized edge-detection system.\textsuperscript{22} In the reproducibility studies the mean difference and SD of the first and second measurements were calculated and compared with previous studies. The interreader, intrareader, and intersonographer mean difference $\pm$ SD [coefficient of variation (CV) in brackets] between paired measurements was $0.106 \pm 0.05$ mm (CV, 0.15), $0.015 \pm 0.08$ mm (CV, 0.14), and $0.085 \pm 0.09$ mm (CV, 0.19), respectively, which compares favorably with previous reports.\textsuperscript{22,23}

#### Statistical Analysis

Mean carotid IMT and maximal carotid IMT measurements were log-transformed to achieve a Normal distribution. Significant covariates were then determined by linear regression using MINITAB; the adjusted residuals from these regressions were used in the genetic analysis. The heritability of log, maximal carotid IMT and log, mean carotid IMT was estimated using Multipoint Engine for Rapid Likelihood Inference (MERLIN).\textsuperscript{24}

A 2-stage approach was used to investigate the association between the IL-6 to $-174 G/C$ polymorphism and carotid IMT. In the first stage of the analysis, adjusted log, carotid IMT was analyzed for an association with the genotype in the entire population by ANOVA. In the second stage, a family-based test of genetic association was performed to rule out population stratification as a cause for any observed association. Identity-by-descent vectors for the polymorphism were calculated using MERLIN,\textsuperscript{24} and family-based tests of association of adjusted log, carotid IMT with the typed marker were carried out using a variance components approach in Quantitative Transmission Disequilibrium Test (QTD T).\textsuperscript{25}

#### Meta-Analysis

The results of this study were considered in the context of a meta-analysis of the available evidence from published studies of the association of the IL-6 to $-174 G/C$ polymorphism and carotid IMT. Relevant studies were sought by searches of the MEDLINE database, with various combinations of key words (eg, gene, carotid IMT, and IL-6 gene); this strategy was supplemented by review of the reference lists of relevant articles. Studies were eligible for inclusion in this meta-analysis if carotid IMT was measured as a continuous trait and if the proportions of the GG, GC, and CC genotypes could be extracted from the report. Two investigators (B.M.M. and P.J.A.) independently reviewed study eligibility and extracted data. The meta-analysis was performed by expressing the phenotypic means of each genotype as deviations from the study means. The results of the studies were pooled to derive the overall means, with weighting by the numbers in each group, and approximated SEs using an estimate of SD of 0.15, a typical value of the within-genotype SD. This approach is a variant of the method of Glass,\textsuperscript{26} which assumes equal within-group variance across the studies. Heterogeneity was assessed by comparing the genotype frequencies between the studies using the $\chi^2$ test.

#### Results

There were 854 members of 224 families who had acceptable carotid IMT measurements, 823 of whom had both phenotype and genotype information available for analysis. There were 99 scanned subjects who were excluded because of poor scan quality. Characteristics of the 854 subjects with carotid IMT measurements are shown in Table 1. Eighty percent of families comprised between 2 and 7 members in 2 generations. The average age of the older generation was $59.2 \pm 8.0$ (years $\pm$ SD), whereas the younger generation had an average age of $35.1 \pm 8.5$. Additional information about family structure is available in Tables I and II, online only at http://www.strokeaha.org.

The maximal rather than the mean carotid IMT was used as the principal variable after preliminary analyses showed highly similar results for the 2 variables, which were highly correlated ($r=0.98$). The median maximal carotid IMT values of 0.858 mm in men and 0.803 mm in women were within the normal population range ($0.36$ to $1.07$ mm).\textsuperscript{27}
The significant covariates for loge maximal carotid IMT were age, sex, body mass index, and physical exercise (coded as a binary variable, none versus some regular exercise). They were all highly significant, \( P < 0.001 \) for the first 3 and \( P = 0.002 \) for exercise. These covariates accounted for 37.7% of variability in loge maximal carotid IMT. After adjustment for covariates, the heritability of loge maximal carotid IMT was 24% (\( P = 0.0002 \)).

The genotype frequencies did not differ significantly from Hardy-Weinberg proportions. Among the 823 subjects with genotype and phenotype information, the mean adjusted loge maximal carotid IMT measurements were \(-0.004 (\pm 0.018 \text{ SE})\) in 265 individuals with GG genotype, \(-0.007 (\pm 0.010 \text{ SE})\) in persons with GC genotype (\( n = 422 \)), and \(0.036 (\pm 0.013 \text{ SE})\) in those homozygous for the C allele (\( n = 136 \)), suggesting that the C allele was behaving in a recessive fashion to increase carotid IMT (Figure 1). Under a recessive model (GC + GG versus CC), there was significant evidence of an association between the genotype and adjusted loge max IMT (\( F = 5.469; 1 \text{ and } 821 \text{ degrees of freedom}; P = 0.02 \)). Family-based analyses using QTDT found no evidence for population stratification as the cause for the association (\( \chi^2 = 0.469; P = 0.4934 \)). A test for total evidence of association in QTDT, which included dominance effects, gave support for the association between the genotype at the IL-6 to -174 G/C polymorphism and adjusted loge maximal carotid IMT (\( \chi^2 = 5.73; P = 0.05 \)).

The CC genotype of the IL-6 to -174 G/C polymorphism was associated with a 4.8% increase in adjusted loge maximal carotid IMT (95% CI, 0.5 to 8.3%); genotype explained 0.6% of the observed variation in the trait. This is equivalent to 2.5% of the heritable component.

Four other studies to date have examined the association between carotid IMT and the IL-6 to -174G/C polymorphism.\(^{10,13-15}\) The 2 smaller studies each investigated <100 individuals, and the 2 larger studies each investigated \( \geq 1000 \) individuals. We found significant differences in genotype frequencies between the studies. The 2 larger studies\(^{14,15}\) had allele frequencies of 0.57 and 0.59, respectively, for the common G allele that were similar to our study (0.58) and other European populations.\(^{28,29}\) However, the 2 smaller studies had significantly different allele frequencies: the study by Rauramaa et al\(^ {10}\) of middle-aged Finnish men showing a much lower G allele frequency of 0.41, and the study by Rundek et al\(^ {13}\) of an American population of diverse ancestral backgrounds showing a significantly higher G allele frequency of 0.8 (\( \chi^2 = 19.88, P = 0.001 \) and \( \chi^2 = 27.69, P = 0.001 \), respectively, for heterogeneity between these and the 3 larger studies).

When the 3 larger studies (including the present study) were combined, the overall weighted mean carotid IMT for the CC genotype (0.0198 ± 0.0068 SE) was significantly greater than...
the mean for the combined GG+GC genotypes \((-0.0039 \pm 0.0038 \text{ SE}; P=0.0014)\), indicating that the C allele was acting in a recessive manner to increase carotid IMT (Table 2). The 2 smaller studies were not included in the meta-analysis because of the unexplained heterogeneity in genotype frequencies.

Given that the previous large studies\(^{14,15}\) suggested a recessive effect of CC versus GG+GC and the SD of adjusted log IMT 0.204, then the present study has an 80% power of detecting a difference in the groups of \(\approx 0.05\) in adjusted log IMT values at the 5% significance level, which translates to a 5% difference in adjusted IMT values.

### Discussion

We found that the size of the genetic contribution to carotid IMT was modest, with a polygenic heritability of 24% \((P=0.0002)\). The estimate is in the lower range of findings of the studies of the heritability of carotid IMT in European, American, and Chinese populations.\(^3\)–\(^9\) Our study may provide a more reliable estimate of heritability because of its large size and its ascertainment strategy favoring large nuclear and extended pedigrees.\(^30\) Previous heritability studies have contained smaller numbers of families,\(^3,6,7\) sibling pairs,\(^3,9\) or twins.\(^5,8\) Heritability estimates that are based solely on sibling or twin analyses could be inflated, because such data do not account for either dominance or correlated environmental factors.\(^30\) We have also carried out an extensive correction both for major demographic covariates (e.g., age and sex) and for causative factors for atherosclerosis, which both have genetic (eg, body mass index) and cultural (eg, physical exercise) transmission; these together accounted for nearly 40% of the interindividual variability in carotid IMT. It remains possible, however, that the heterogeneity between the estimates of heritability in published studies may in large part be related to differences in study design (including ascertainment criteria) or to population-specific factors.

This is the first large family study to show association of genotype at a functional polymorphism of the IL-6 locus with carotid IMT. The C allele of the IL-6 to \(-174G/C\) polymorphism was associated with a 4.8% greater maximal carotid IMT in a recessive fashion. The entire contribution of the IL-6 locus to the variability of atherosclerosis was small \((R^2=0.6\%\)), and the statistical significance level of the association, calculated either using ANOVA or QTDT, was borderline even in this large family collection. The meta-analysis of the large studies of association between carotid IMT and the IL-6 to \(-174G/C\) polymorphism, however, confirmed a significant association of the CC genotype with carotid IMT, with a high degree of statistical significance. Subgroup analyses of 2 of the larger population-based studies showed that the C allele was associated with increased IMT among alcohol abusers\(^14\) and in older individuals,\(^15\) but no heterogeneity between strata of alcohol consumption, between younger and older individuals, or between hypertensives and nonhypertensives was observed in the present study (data not shown).

Unlike the other studies that have examined this question,\(^10,13\)–\(^15\) our study was designed a priori to maximize the chances of detection of genes that influence cardiovascular risk. In particular, the family design with extreme ascertainment for a trait strongly related to cardiovascular risk substantially increases the power over random sampling for the detection of genes of small effect.\(^31\) This ascertainment scheme resulted in the selection of individuals with a 33% higher mean carotid IMT than in previous studies of the general population\(^9\) or the other large studies of the genetic association of this question.\(^14,15\) This approach may have given our study greater power to reliably quantify the modest association of the IL-6 \(-174G/C\) polymorphism with carotid IMT than has been possible with previous study designs.

The potential disadvantage of our ascertainment strategy is that, in the presence of significant interaction between hypertension and the IL-6 gene on the determination of carotid IMT, bias could be introduced. However, the IL-6 genotype was not associated with hypertension, and there was no heterogeneity between hypertensives and nonhypertensives with respect to the effect of the IL-6 polymorphism on IMT. Also, the meta-analysis result, which included studies in populations not ascertained through hypertension, reinforced the result in our families.

In conclusion, about a quarter of the variability in carotid IMT is explained by genetic factors. One of the genes that influence carotid IMT is the IL-6 gene, a functional polymorphism of which is responsible for 0.6% of the variability of the adjusted log IMT values and 2.5% of the heritable component of carotid IMT. Our findings in this large population of hypertensive families and meta-analysis of published studies support the notion that, although regulatory variants of inflammatory genes may be significant factors in susceptibility to atherosclerosis, their effect is likely to be of small size.

### Acknowledgments

We are grateful to the families who contributed to this project. B.K. designed the family collection strategy, ascertainment and collected the

### Table 2. Meta-Analysis of the Studies of Association between Carotid IMT and IL-6 \(-174G/C\) Polymorphism

<table>
<thead>
<tr>
<th>Study</th>
<th>GG Mean (No.)</th>
<th>GC Mean (No.)</th>
<th>CC Mean (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rauramaa 2000(^{10})</td>
<td>0.1362 (19) ±0.0344</td>
<td>-0.0738 (38) ±0.0243</td>
<td>0.0062 (35) ±0.0253</td>
</tr>
<tr>
<td>Rundeck 2002(^{13})</td>
<td>0.0279 (47) ±0.0219</td>
<td>-0.0421 (19) ±0.0344</td>
<td>-0.1021 (5) ±0.0671</td>
</tr>
<tr>
<td>Jerrard-Dunne 2003(^{14})</td>
<td>-0.0036 (320) ±0.0084</td>
<td>-0.0036 (500) ±0.0067</td>
<td>0.0164 (180) ±0.0112</td>
</tr>
<tr>
<td>Chapman 2003(^{15})</td>
<td>-0.0073 (380) ±0.0077</td>
<td>0.0027 (556) ±0.0064</td>
<td>0.0077 (171) ±0.0115</td>
</tr>
<tr>
<td>Present study</td>
<td>-0.0056 (265) ±0.0092</td>
<td>-0.0092 (422) ±0.0073</td>
<td>0.0395 (136) ±0.0129</td>
</tr>
<tr>
<td>Weighted mean (3 large studies)</td>
<td>-0.0056 (965) ±0.0048</td>
<td>-0.0028 (1478) ±0.0039</td>
<td>0.0198 (487)* ±0.0068</td>
</tr>
</tbody>
</table>

\*\(P=0.0014\) (GG+GC vs CC).
families, and executed the initial phenotyping for hypertension and blood collection for DNA.

References


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