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Published in:
Circulation Research
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*Circ. Res.* 2006;99;102-108; originally published online Jun 15, 2006;
DOI: 10.1161/01.RES.0000232324.87983.4b

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Abstract—Caspase-1 processes the interleukin (IL)-1β and IL-18 inactive precursors to the biologically active cytokines that are known to have proatherogenic effects. The present study investigated the genetic variability of the CASP1 gene and plasma levels of caspase-1 in relation to cardiovascular risk. In Europeans, 3 tag SNPs captured 4 common haplotypes of the CASP1 gene. Among these, the A<sup>66</sup> allele of the G+7/in6A polymorphism was less frequent in 246 cases with myocardial infarction and a parental history of disease than in 253 controls free of familial history of disease (0.13±0.02 versus 0.20±0.02; P=0.005). However, in a larger case/control study (n=1774), these effects are borderline restricted to the UK population. In a prospective cohort of 1168 patients with coronary artery disease followed up during a median period of 6.0 years, the A<sup>66</sup> allele exhibited a borderline association with future cardiovascular death (hazard ratio [HR]: 0.64, 0.41 to 1.01; P=0.053) and was associated with lower serum IL-18 levels (P=0.014). Baseline caspase-1 levels in the top quartile of the distribution were predictive of cardiovascular deaths (HR=3.62, 1.81 to 7.27; P=0.0003 compared with the bottom quartile). Finally, in vitro assays of allelic imbalance showed that the CASP1 haplotype carrying the A<sup>66</sup> allele was associated with a lower mRNA expression. These results indicate that caspase-1 levels are predictive of future cardiovascular death in patients with coronary artery disease. The role of CASP1 genetic variations in the susceptibility to myocardial infarction requires further investigation. (Circ Res. 2006;99:102-108.)

Key Words: caspase-1 ■ inflammation ■ prognosis ■ coronary disease ■ genetics

Caspases are a family of intracellular cysteine proteases that include at least 14 members that have been identified in mammals to date. Caspases play an essential role in apoptosis, but a subfamily comprising caspase-1, -4, and -5 are primarily involved in the regulation of inflammatory processes. Caspase-1, the best characterized of inflammatory caspases, was originally identified on the basis of its proteolytic activity for cleaving the inactive interleukin (IL)-1α precursor into the mature cytokine. This role was further confirmed by the impaired production of mature IL-1β in caspase-1-deficient mice. Caspase-1 is also responsible for the processing of IL-18, a cytokine that shares several structural features with the IL-1 family. The production of mature IL-18 requires cleavage of its precursor pro–IL-18 at a site similar to the cleavage site of pro–IL-1β.

Increasing evidence has accumulated from animal, experimental, and epidemiological data that IL-1 and IL-18 are involved in both development of atherosclerosis and plaque vulnerability as well as myocardial dysfunction as a consequence of ischemia/reperfusion injury. Given the critical role of caspase-1 in the production of these 2 cytokines, this enzyme may play a key role in the proatherogenic effects mediated by IL-1β and IL-18. Several lines of evidence support such a role. Caspase-1–deficient mice have a reduced production of interferon-γ, a proatherogenic factor whose induction by T cells and macrophages is stimulated by IL-18. Caspase-1 has been colocalized with apoptotic macrophages in sites of plaque rupture. Finally, inhibition of caspase-1 has been shown to reduce myocardial ischemic dysfunction in models of ischemia/reperfusion via an inhibition of IL-18 and IL-1β.

As previously shown, circulating IL-18 levels were strongly predictive of future cardiovascular events. Furthermore, polymorphisms in the IL18 gene might contribute to the predisposition to complications of coronary artery disease (CAD). Given the impact of caspase-1 in the production of IL-18, the present study aimed to investigate whether baseline caspase-1 levels and polymorphisms of the CASP1 gene might influence cardiovascular risk. We sequenced the CASP1 gene and genotyped all single nucleotide

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DOI: 10.1161/01.RES.0000232324.87983.4b

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polymorphisms (SNPs) in the SIPLAC study, a case/control study of myocardial infarction (MI). Three tag SNPs were then studied in relation to future cardiovascular death in the prospective AtheroGene cohort of CAD patients, and the most interesting tag SNP was further genotyped in the ECTIM study, a large case/control study of MI. Finally, functional assays were performed to detect potential differences in allelic expression of the CASP1 gene.

Materials and Methods

Study Populations

AtheroGene

The AtheroGene study is based on a prospective cohort of CAD patients recruited at the occasion of a diagnostic coronary angiography (Figure 1).20 A priori inclusion criterion was the presence of a diameter stenosis of >30% in at least 1 major coronary artery. The initial cohort was recruited between November 1996 and June 2000 and followed up for a median period of 6.0 (maximum 7.6) years. Stable (SAP) and unstable (UAP) angina pectoris were coded by Braunwald classification. Baseline IL-18 measurements and DNA were available for 1178 patients, of whom 106 died from a cardiovascular cause. Information about the cause of death was obtained by hospital or general practitioner. Extension of recruitment until February 2004 resulted in the inclusion of an additional 1294 patients, of whom 26 died from a cardiovascular cause. DNA, but not IL-18, was available in this extension cohort.

Because plasma samples of the early recruitment period tended to have been exhausted, caspase-1 was measured in patients recruited from June 1999 (n=1894). They were followed up for a median period of 2.5 (maximum 5.0) years and 71 died from a cardiovascular cause. There was an overlap of 450 patients in whom both caspase-1 and IL-18 were determined.

Healthy control subjects (n=468) were recruited either from the offices of general practitioners in the course of a routine check-up or by newspaper announcement. Caspase-1 was measured in 60% of controls for whom plasma was available.

Study participants had German nationality, were inhabitants of the Rhein-Main area, and were of European origin. Each participant gave written informed consent.

ECTIM and SIPLAC

The ECTIM study is a case/control study of MI conducted in the United Kingdom and France.21,22 Patients (35% females in the UK, only males in France) aged 25 to 64 years (for males) and 25 to 69 years (for females) were recruited from the WHO MONICA Project registers23 in Belfast and Glasgow (UK) and Lille, Strasbourg, and Toulouse (France). Age- and sex-matched controls were recruited from the populations of the areas covered by the registers. DNA was available for 1143 cases and 1133 controls.

The SIPLAC study is a substudy of ECTIM including highly contrasted samples of cases with MI and a parental history of MI (n=246) and controls without CAD and without parental history of MI (n=253) from the United Kingdom. This study provides us with a tool for selecting, among all SNPs discovered by molecular screening, the subset of those that will be further genotyped in larger studies, after exclusion of rare (≤0.02) and redundant polymorphisms. It is also intended to rapidly detect MI-associated polymorphisms that will require further replication.

Laboratory Methods in the AtheroGene Study

Blood was drawn under standardized conditions before coronary angiography was performed, and samples were stored at −80°C. Plasma caspase-1 (R&D Systems) and serum IL-18 (MBL Co Ltd) were measured using the ELISA technique according to the instructions of the manufacturer. C-reactive protein (CRP) was determined by a highly sensitive (hs), latex particle-enhanced immunosassay (Roche Diagnostics, Mannheim, Germany). Serum lipid levels were determined immediately by routine methods.

SNP Discovery in the CASP1 Gene

We sequenced all exons, up to 100 bp, of exon/intron junctions and up to 1 kb of 3’- and 5’-flanking sequences. Sequencing was performed in 31 pools of 2 MI patients (62 individuals) using a 16-capillary ABI-PRISM 3100 system and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems [ABI], Warrington, UK). Sequences were compared using the ABI-PRISM SeqScape software version 2.0.

Genotyping

SNPs detected by sequencing were genotyped in the SIPLAC study by allele-specific oligonucleotide hybridization. Two tag SNPs (G+7/in6A and G+1034C) were selected for genotyping in the AtheroGene cohort plus a third SNP located in intron 8 (T+132/in8C), which, after consultation of the HapMap database (http://www.hapmap.org), appeared necessary to capture all common haplotypes of the CASP1 gene. Finally, the G+7/in6A SNP was genotyped in the ECTIM study. Genotyping in AtheroGene and ECTIM was performed using the 5’ nuclelease assay with TaqMan probes (see the expanded Materials and Methods in the online data supplement, available at http://circres.ahajournals.org). Primer and probe sequences, as well as amplification conditions for genotyping, can be found at our website, GeneCanvas (http://www.genecanvas.org).

Statistical Analysis

Departure from Hardy–Weinberg equilibrium was tested by χ² with 1 df. Association of polymorphisms with case/control status was tested by χ² analysis and with prospective outcome by Cox proportional-hazards regression analysis. Different models were successively fitted, first adjusted for age and sex, then additionally adjusted for cardiovascular risk factors (body mass index, diabetes, hypertension, smoking, HDL-cholesterol, triglycerides, acute coronary syndrome, number of diseased vessels, history of MI, statin, and β-blocker intake), and finally adjusted for IL-18 levels. In all prospective analyses, the primary end point was cardiovascular death, and patients who died from other causes were censored at the time of death. Association of baseline caspase-1 levels, in quartiles, with cardiovascular mortality was tested by Cox analysis adjusted in the same way as for analyses with genotype. Association of polymorphisms with IL-18 and caspase-1 levels was tested by the GLM procedure adjusted for age, sex, and case/control status, and homogeneity of association between cases and controls was tested by introducing an interaction term in the model. IL-18 and caspase-1 were log-transformed to remove positive skewness and geometric means (95% confidence interval [CI]) are given.

Figure 1. Variables measured in the different study populations. The CASP1 G +7/in6A SNP was genotyped in all studies.
Linkage disequilibrium (LD) and haplotype analyses were performed using the THESIAS software, available at http://www.genecanvas.org. Haplotypic effects, expressed as mean quantitative effects or as odds ratios (ORs), were estimated by comparison to the most frequent haplotype. A global test of association between haplotypes and the phenotype was performed by a likelihood ratio test. The homogeneity of effects associated with haplotypes carrying the \( A^{ine} \) allele was tested by introducing a constraint on the parameters \( \chi^2 \) with 1 df. Except for haplotype analyses, all analyses were performed with the SAS software version 8.01 (SAS Institute Inc., Cary, NC). \( P < 0.05 \) was considered to be significant.

**Allelic Imbalance Assays**

Differential allelic expression of the \( CASP1 \) gene was tested by the method of allelic imbalance, which compares the ratio of cDNAs to that of genomic DNAs in individuals heterozygous for a transcribed method of allelic imbalance, which compares the ratio of cDNAs to that of genomic DNAs in individuals heterozygous for a transcribed polymorphism (expanded Materials and Methods). cDNA and genomic DNA from 11 individuals heterozygous for the L256L (C/T) polymorphism were subjected to the same reaction of allelic discrimination using the TaqMan technology. Each sample was submitted to 2 different RT-PCR, and the resulting cDNAs were amplified in duplicates. The regression line equation from the genomic DNA samples was calculated, and, for each cDNA sample, the deviation from this theoretical line was assessed. For each individual, the log (ratio) of the observed to the expected value was averaged over the 4 repetitions. The mean of the 11 values was compared by \( t \) test to the expected value of 0 under the null hypothesis of no allelic imbalance.

**Real-Time RT-PCR Assays**

mRNA expression levels in LCLs were compared between individuals with different genotypes for the L256L (C/T) polymorphism by real-time PCR quantification (expanded Materials and Methods). Twenty-two homozygotes for the C allele, 9 heterozygotes, and 2 homozygotes for the T allele were analyzed in duplicate, and the mean of the 2 measurements was taken. Rare homozygotes were pooled with heterozygotes for final comparison. The mean mRNA levels between CC subjects and T carriers were compared by \( t \) test.

**Results**

**Sequencing of the \( CASP1 \) Gene and Genotyping in the SIPLAC Study**

Nineteen polymorphisms were detected by sequencing (Figure 2), which were all genotyped in the SIPLAC study, except 3 of them, which were very rare (A + 109/in5G, C + 92/in7T, and −43/in7/D, each observed in 1 of 124 chromosomes). There was a block of 14 SNPs in complete association covering the whole gene sequence. Only 2 SNPs were outside of this block, a nonsynonymous SNP (R240Q) that was present in only 2 subjects and not further studied and 1 SNP located in the 3’ region (G + 1034C). The G + 7/in6A SNP was selected for representing the block in complete association.

In the SIPLAC study, the G + 7/in6A and G + 1034C SNPs were in almost complete LD (\( D’ = 0.97 \)). The \( A^{ine} \) allele was represented less in MI cases than in controls (0.13 ± 0.02 versus 0.20 ± 0.03 in Belfast, 0.12 ± 0.02 versus 0.19 ± 0.03 in Glasgow; \( P = 0.005 \) for the case/control difference adjusted for center). The G + 1034C SNP was not associated with MI risk (supplemental Table I).

**CASP1 Polymorphisms and CAD Risk in the AtheroGene Study**

Baseline characteristics of cases and controls in the initial AtheroGene study are shown in Table 1. Cases had increased levels of CRP, triglycerides, and a higher ratio of LDL- to HDL-cholesterol. The lower levels of LDL in patients was likely attributable to the high prevalence of lipid-lowering treatment. IL-18 levels were comparable in controls and CAD patients as a whole. However, there was a trend toward increased IL-18 levels in acute MI by comparison to UAP and SAP (66.0 versus 60.9 and 59.7 pg/mL; \( P = 0.11 \)). In the subset of subjects having both IL-18 and caspase-1 measurements, caspase-1 levels were higher in cases than in controls (Table 1).

The G + 7/in6A, T + 132/in8C, and G + 1034C SNPs were genotyped in the initial AtheroGene study. Because of strong LD, they generated only 4 common haplotypes, \( G^{T/in7}T^{in7}G^{+1034} \), \( G^{C/in6}C^{+1034} \), \( A^{T/in7}T^{in7}A^{+1034} \), and \( A^{T/in7}T^{in7}C^{+1034} \), with frequency in controls of 0.54, 0.28, 0.06, and 0.12, respectively. The \( A^{ine} \) allele frequency was similar in healthy German controls to that observed in controls from the United Kingdom (0.18 ± 0.01 versus 0.19 ± 0.02; \( P = 0.45 \)). There was no difference of the \( A^{ine} \) allele frequency between CAD patients as a whole and controls (0.17 ± 0.01 versus 0.18 ± 0.01; \( P = 0.75 \)). However, the \( A^{ine} \) allele frequency was significantly decreased in patients with acute MI (0.11 ± 0.02; \( P = 0.042 \)), whereas it did not differ in patients with SAP (0.17 ± 0.01; \( P = 0.79 \)) or UAP (0.19 ± 0.02; \( P = 0.60 \)) from the frequency.

![Figure 2. Polymorphisms of the CASP1 gene identified by sequencing. Hatched boxes indicate UTRs. Polymorphisms in 5’ and 3’ are numbered upstream from the start of translation and downstream from the stop codon, respectively. Polymorphisms in exon/intron junctions are numbered downstream from the end of the preceding exon or upstream from the start of the next exon. Reference SNP id (rs) are given when possible. Boxed polymorphisms are in complete association. Polymorphisms genotyped in the AtheroGene study are in bold (including the T + 132/in8C selected from HapMap).](image-url)
observed in controls (supplemental Table II). The T+132/in8C and G+1034C SNPs were not associated with clinical status (supplemental Table II). Haplotype frequencies did not differ between cases as a whole and controls ($P=0.87$).

**CASP1 Polymorphisms and Circulating IL-18 Levels in the AtheroGene Study**

The G+7/in6A SNP was associated with serum IL-18 levels, the A<sup>1034</sup> allele being associated with a lowering effect in a fairly additive fashion (Table 2). The T+132/in8C and G+1034C SNPs were not associated with IL-18 levels. Haplotype analysis was performed in cases and controls pooled because there was no significant heterogeneity between groups. By reference to the most frequent haplotype (G<sup>1034C</sup>T<sup>132/in8C</sup>), haplotype G<sup>1034C</sup>A<sup>in6T</sup>in8C<sup>1034</sup> had no significant effect on IL-18 levels ($P=0.97$), whereas haplotypes A<sup>1034T</sup>in8C<sup>1034</sup> and A<sup>1034T</sup>in8C<sup>1034</sup>C<sup>1034</sup> had similar lowering effects ($P=0.48$ for the homogeneity of effects), suggesting that neither the T+132/in8C nor the G+1034C SNP had an individual effect and that only the G+7/in6A SNP was associated with IL-18 levels (Figure 3).

**CASP1 Polymorphisms and Cardiovascular Mortality in the AtheroGene Study**

In the initial AtheroGene cohort, there was a trend toward a lower risk of cardiovascular deaths during follow-up in carriers of the A<sup>1034</sup> allele than in G<sup>1034C</sup> homozygotes, although the association did not reach significance (age- and sex-adjusted hazard ratio [HR]: 0.64, 0.41 to 1.01; $P=0.053$). Kaplan–Meier curves of survival according to genotype are shown in Figure 4. Adjustment for cardiovascular risk factors did not modify the relationship (HR=0.65, 0.41 to 1.03; $P=0.07$), nor did additional adjustment for IL-18 (HR=0.68, 0.43 to 1.08; $P=0.10$). No significant association was observed with the T+132/in8C or the G+1034C SNP (supplemental Table III).

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**TABLE 1. Baseline Characteristics of Cases With Coronary Artery Disease and Control Subjects in the Initial AtheroGene Study**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases with CAD (n=1178)</th>
<th>Controls (n=468)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61.6±0.3</td>
<td>59.9±0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Males, %</td>
<td>74.4</td>
<td>72.6</td>
<td>0.46</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.2±0.1</td>
<td>26.6±0.2</td>
<td>0.004</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>13.5</td>
<td>10.7</td>
<td>0.13</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>16.6</td>
<td>1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>72.9</td>
<td>28.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Statin, %</td>
<td>35.7</td>
<td>7.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>β-blocker, %</td>
<td>59.3</td>
<td>9.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACE-inhibitor, %</td>
<td>46.9</td>
<td>8.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>219.0±1.3</td>
<td>238.4±2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dL</td>
<td>140.3±1.1</td>
<td>155.4±1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>48.7±0.4</td>
<td>59.9±0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>3.07±0.03</td>
<td>2.76±0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>145.9 (141.8–150.2)</td>
<td>119.8 (114.5–125.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>5.3 (4.9–5.7)</td>
<td>1.7 (1.4–1.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-18, pg/mL</td>
<td>60.4 (59.0–61.8)</td>
<td>60.8 (58.6–63.1)</td>
<td>0.74</td>
</tr>
<tr>
<td>Caspase-1, pg/mL</td>
<td>50.8 (48.5–53.1)</td>
<td>45.0 (42.4–47.7)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Categorical variables are presented as percentages and continuous variables as age- and sex-adjusted means±SE, except for *log-transformed variables, which are presented as adjusted geometric means (95% CI). †Caspase-1 was measured in 450 cases of the initial cohort and 276 controls

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**TABLE 2. Association of CASP1 Polymorphisms With IL-18 and Caspase-1 Levels in Cases and Controls of the AtheroGene Study**

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>IL-18 (pg/mL)</th>
<th>Caspase-1 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>p</td>
</tr>
<tr>
<td>G+7/in6A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>1171</td>
<td>60.9 (59.2–62.7)</td>
</tr>
<tr>
<td>Controls</td>
<td>468</td>
<td>62.4 (59.9–65.0)</td>
</tr>
<tr>
<td>T+132/in8C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>1151</td>
<td>59.8 (57.8–61.8)</td>
</tr>
<tr>
<td>Controls</td>
<td>468</td>
<td>60.6 (57.2–63.0)</td>
</tr>
<tr>
<td>G+1034C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>1178</td>
<td>60.6 (58.9–62.2)</td>
</tr>
<tr>
<td>Controls</td>
<td>467</td>
<td>62.3 (59.9–64.7)</td>
</tr>
</tbody>
</table>

Age- and sex-adjusted geometric means (and 95% CI) are shown, and tests are performed on log-transformed values. *Test of main effect, †test of homogeneity between cases and controls.
To increase the power of the survival analysis, the G7/in6A SNP was analyzed in the extension cohort. When combining both cohorts, the HR associated with carriage of the Ain6 allele, before and after adjustment for cardiovascular risk factors, was 0.66 (0.44 to 0.99; \( P = 0.04 \)) and 0.68 (0.45 to 1.03; \( P = 0.07 \)), respectively.

**Plasma Caspase-1 Levels and Cardiovascular Mortality in the AtheroGene Study**

When considering all subjects having a caspase-1 determination (Figure 1), higher levels were observed in CAD patients than in healthy controls, but this difference was mainly attributable to an increase in MI patients (age- and sex-adjusted mean levels: 59.3, 52.8, 51.9, and 51.4 pg/mL in MI, UAP, SAP, and control groups, respectively; \( P < 0.0001 \)). Caspase-1 correlated positively with CRP (\( r = 0.19; \ P < 0.001 \)), but the association with IL-18 was rather weak (\( r = 0.14; \ P = 0.001 \)) despite their biological link.

Figure 5 displays the Kaplan–Meier curves for survival from cardiovascular death according to caspase-1 quartiles. HRs estimated from different Cox models are given in supplemental Table IV. All models indicated an increased risk in patients in the top quartile of caspase-1 levels compared with those in the bottom quartile, whereas patients in the intermediate quartiles were not at higher risk. The age- and sex-adjusted HR (95% CI) in the top quartile was 3.62 (1.81 to 7.27; \( P = 0.0003 \)). Adjustment for classical risk factors hardly modified the association (HR = 2.89, 1.42 to 5.88; \( P = 0.003 \)). Further adjustment for CRP levels slightly weakened the association (HR = 2.32, 1.13 to 4.79; \( P = 0.03 \)).

**CASP1 Polymorphisms and Plasma Caspase-1 Levels in the AtheroGene Study**

There was a borderline association of the G7/in6A and the G1034C SNPs with plasma caspase-1 levels, the minor allele of each SNP being associated with a lowering effect (\( P = 0.066 \) for the significance of each polymorphism, Table 2). By reference to the G\(^{\text{in6G}}\)1034 haplotype, haplotypes A\(^{\text{in6A}}\)G\(^{\text{1034}}\) and A\(^{\text{in6C}}\)C\(^{\text{1034}}\) had similar lowering effects (\( P = 0.65 \) for the homogeneity of effects), indicating that the G\(^{\text{1034C}}\) SNP had no effect by itself. The T\(^{\text{132/in8C}}\) SNP was genotyped only in a subset of subjects having a caspase-1 measurement and there was no significant association.

**CASP1/G\(^{\text{+7/in6A}}\) Polymorphism and MI Risk in the ECTIM Study**

Because of the suggestive evidence of an association of the G\(^{\text{+7/in6A}}\) SNP with MI risk both in SIPLAC and AtheroGene, we further genotyped this SNP in the whole ECTIM study (Figure 1). In the whole population from the United Kingdom, encompassing SIPLAC, the association was weakened, the A\(^{\text{in6A}}\) allele frequency being 0.146±0.009 in cases and 0.170±0.010 in controls, respectively (\( P = 0.07 \)). By contrast, there was no case/control difference of allele frequency in France (0.175±0.014 versus 0.161±0.013; \( P = 0.46 \)) (test of homogeneity between countries; \( P = 0.10 \)). When stratifying according to parental history of MI, there was no significant interaction in either country (supplemental Table V).

**Functional Assays**

We further examined whether the 2 haplotypes defined by the block containing the G\(^{\text{+7/in6A}}\) SNP were associated with a
difference in gene expression levels. The exonic polymorphism taken for allelic imbalance assays was the coding SNP L256L (C/T), which belongs to the block (Figure 2). Expression of allelic transcripts in 11 heterozygotes are shown in Figure 6. The regression line equation predicted from the genomic DNA samples was associated with an \( R^2 \) of 98\%, compared with 100\%, for a perfect equimolar ratio between the 2 alleles. Most values for the cDNA samples were situated above the regression line, suggesting a higher expression level associated with the C allele than with the T allele (mean difference of the observed to expected values : 0.31±0.12; \( P=0.028 \)).

We confirmed these results by the quantification of mRNA levels by real-time RT-PCR. Higher expression levels were found in individuals homozygous for the C allele (1.91±0.34, \( n=22 \)) than in carriers of the T allele (0.53±0.48, \( n=11 \)) (\( P=0.02 \)).

**Discussion**

Caspase-1 belongs to the subfamily of inflammatory caspases, also comprising caspase-4 and -5, that share similar biological functions in the regulation of inflammatory processes.\(^5\) In particular, all 3 caspases can cleave pro–IL-18 to generate the biologically active IL-18, although caspase-1 is the most efficient in this process.\(^6\)\(^7\) The genes coding for these evolutionarily-related caspases are located in a cluster on chromosome 11q22.2 to 22.3 and present more than 50% of sequence homology.\(^1\) Besides the \( \text{CASP4} \) and \( \text{CASP5} \) genes located downstream, we identified several regions located upstream and presenting more than 70% of homology with the \( \text{CASP1} \) gene sequence. These sequences are presumably those that were previously identified and considered to be pseudogenes, because none of these encoded a functional cysteine protease.\(^26\)

The \( \text{CASP1} \) gene exhibited a pattern of reduced haplotype diversity, with only 4 haplotypes captured by 3 tag SNPs. We investigated whether this block might extend beyond the \( \text{CASP1} \) gene itself by examining the HapMap data. In Europeans, the block stretched over 32 kb but did not include the \( \text{CASP4} \) and \( \text{CASP5} \) genes, therefore excluding the possibility that polymorphisms in these genes might be responsible for the observed associations.

Our study presented a comprehensive investigation of the role of caspase-1 in susceptibility to CAD and its complications. However, it failed to provide definite conclusions. Our initial results provided several arguments supporting an etiologic role of caspase-1: (1) the \( \text{A}^{\text{66}} \) allele of the \( \text{CASP1} \) gene was associated with a lower risk of MI in 2 different case/control studies, SIPLAC, and AtheroGene; (2) the same allele exhibited a borderline association with prospective cardiovascular death in AtheroGene; (3) plasma caspase-1 was elevated in patients with acute MI and appeared as a biomarker predictive of cardiovascular mortality in CAD patients; (4) circulating IL-18 levels were decreased in individuals carrying the \( \text{A}^{\text{66}} \) allele; and (5) the \( \text{CASP1} \) haplotype carrying the \( \text{A}^{\text{66}} \) allele was associated with a lower gene expression, supporting the existence of a functional polymorphism within the \( \text{CASP1} \) gene.

Cases in SIPLAC were young survivors of MI with a parental history of disease recruited in the United Kingdom. They were compared with controls selected on the absence of familial and personal histories of MI. Moreover, populations of Northern Ireland and Scotland are among those having the highest prevalence of CAD in Europe.\(^23\) When stratified according population in the entire ECTIM study, the effect was observed in the United Kingdom and was absent in those recruited in France. It is then likely that the combination of these 2 criteria—family history and populations—has led to the selection of individuals genetically more susceptible than the other subjects included in the ECTIM study. However, it cannot be ruled out that, given the multiple hypotheses tested and the lack of replication, the initial associations were attributable to chance. Further studies are required to address this issue.

The genetic effects observed in SIPLAC and AtheroGene were modest and can be viewed only as suggestive of a role of caspase-1 in the etiology and clinical consequences of MI. By linking up the epidemiological and experimental results, it might be speculated that the haplotype carrying the \( \text{A}^{\text{66}} \) allele, which is associated in vitro with a lower CASP1 gene expression, results in a reduced rate of cleavage of pro–IL-18 into the biologically active IL-18, which in turn would confer a protection against the proinflammatory effects of IL-18. Given the complexity of the haplotype block, including at least 14 polymorphisms, it is difficult to predict which among them might be functional. Two of the SNPs are synonymous polymorphisms (T54T and L256L), which are unlikely to have a functional role. Six SNPs are located in exon/intron junctions and might cause alternative splicing that would result in the production of proteins of different sizes. However, we did not detect any difference in total cDNA size among individuals with different genotypes (data not shown). One SNP is located in the 5′ region and might affect gene expression, whereas 5 SNPs are in the 3′ region and might be involved in mRNA stability.

Circulating caspase-1 levels were independently related to future cardiovascular death in CAD patients. This result supports the hypothesis that activation of the caspase-1/IL-18 pathway is involved in the clinical complications of preexisting atherosclerotic disease. In animal models, inhibition of caspase-1 has been shown to reduce ischemia/reperfusion injury.\(^17\)\(^18\) Therefore, increased levels of caspase-1 might reflect individuals at high risk of post-MI complications, rather than early development of atherosclerosis itself. In the same vein, caspase-1 and IL-18 levels were increased in
patients with acute MI but not UAP. The discrepancy might also corroborate the hypothesis that the caspase-1/IL-18 pathway is predominantly activated after MI has occurred.

As an important limitation with regard to the results of the genetic variations, we could not correct for multiple testing. Therefore, these results have to be considered as hypotheses generating and need replication in large population studies. Paradoxically, circulating levels of caspase-1 weakly correlated with those of IL-18, and the CASP1 gene effects were stronger on IL-18 than on caspase-1 itself. This might be explained by the fact that the caspase-1 assay determined the mass and not the activity itself, whereas the IL-18 assay measured a compound of the active form and the IL-18/IL-18-binding protein complex.

In conclusion, the present results indicated that caspase-1 levels were predictive of future cardiovascular death in CAD patients. They also suggested an etiologic role of CASP1 genetic variations in the susceptibility to MI, although this requires further replication.

Acknowledgments

We thank Biosite Inc for determination of caspase-1 levels.

Sources of Funding

The ECTIM study was supported by a grant of the British Heart Foundation (PG93045). The AtheroGene study was supported by a grant from the “Stiftung Rheinland-Pfalz für Innovation,” Ministry for Science and Education (AZ 15202-386261/545), Mainz, Germany; by the MAIFOR grant 2001 from the Johannes Gutenberg-University Mainz, Germany; and by grants from the Fondate de Science and Education (AZ 15202-386261/545), Mainz, Germany. The present study was supported by a grant from the “Stiftung Rheinland-Pfalz für Innovation,” Ministry for Science and Education (AZ 15202-386261/545), Mainz, Germany.

Disclosures

None.

References


