**Aims**

The interleukin 18 (IL-18) gene has a single nucleotide promoter region (−137) G-to-C polymorphism (rs187238) which leads to attenuated transcriptional activity of the gene and to lower production of pro-atherogenic IL-18. The C allele of this polymorphism is associated with a lower risk of sudden cardiac death (SCD). We examined the process by which this polymorphism alters the risk of SCD and coronary artery disease (CAD) by analysing the interactions between this polymorphism and environmental factors.

**Methods and results**

TaqMan 5′ nuclease assay was used to genotype the study population of the Helsinki Sudden Death Study, comprising medicolegal autopsies of 700 men. According to adjusted logistic regression analysis, there was a significant interaction between IL-18 genotype and hypertension impacting on the risk of SCD due to coronary heart disease (CHD) \((P = 0.011)\) and the severity of autopsy-verified CAD \((P = 0.026)\). Among GG homozygotes, hypertension was a major risk factor for SCD due to CHD [adjusted odds ratio (OR) 3.75 with 95% CI 1.78–7.91, \(P < 0.001\)] and hypertension also associated with larger coronary atherosclerotic plaque areas \((P = 0.002)\) and the occurrence of complicated plaques [adjusted OR 8.38 with 95% CI 2.39–29.33, \(P < 0.001\)]. Among C allele carriers, hypertension was not a significant risk factor for CHD-related SCD or CAD and did not associate with the development of coronary atherosclerotic plaques. According to gene expression analysis of atherosclerotic tissue samples obtained from live patients, hypertension also interacted significantly with IL-18 genotype affecting the expression of IL-18 \((P = 0.030)\) mRNA and interferon-γ mRNA \((P = 0.004)\).

**Conclusion**

Hypertension interacts with IL-18 gene promoter −137 G/C polymorphism, affecting the risk of SCD and the development of coronary atherosclerosis.

**Keywords**

Genetics • Sudden cardiac death • Hypertension • Inflammation • Polymorphism • Coronary heart disease

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**Introduction**

Sudden cardiac death (SCD) is a major killer in the developed nations.\(^1\)–\(^3\) For example, according to a report by the US Center of Disease Control (CDC), a total of 462 340 SCDs occurred in the USA in 1999, and 341 780 (74%) of these cases were classified as out-of-hospital deaths.\(^1\) The underlying cause of most SCDs (80%) is pre-existing coronary heart disease (CHD),...
expressed as acute coronary syndrome, myocardial scarring due to a previous infarction or heart failure. The major risk factors for CHD include hypertension, smoking, male sex, obesity, and diabetes. Many of the same factors, such as smoking, hypertension, and sex, also increase the risk of SCD, but on an individual scale the risk of SCD is hard to predict. Even less is known about the specific genetic factors associated with SCD, although family history of SCD, primary cardiac arrest, and myocardial infarction (MI) are known to increase the risk. The problem most likely lies in the complex aetiology of CHD and different phenotypes of SCD. The interactions between traditional risk factors (e.g., hypertension and smoking) and genetic variation may explain individual differences in the predisposition for coronary artery disease (CAD) and SCD.

The human interleukin 18 (IL-18) gene has a common single nucleotide polymorphism (SNP, rs187238) located at the promoter region in position −137 (G/C). We have previously shown that the C allele carriers of this polymorphism have a lower risk for SCD caused by CHD. This polymorphism is in complete linkage disequilibrium with two other SNPs (+1137T/G and +127CT). It decreases the transcriptional activity of the gene and the production of IL-18. IL-18 is a pro-inflammatory and pro-atherogenic cytokine, and it has been directly associated with the development of unstable atherosclerotic plaques. The production of IL-18 leads to the secretion of interferon-γ (IFN-γ) and matrix metalloproteinases (MMPs), factors which are associated with instability in atherosclerotic plaques. The development of unstable plaques increases the risk of acute cardiac events and may also lead to the development of coronary stenosis. Similarly, IL-18 genetic variability and increased plasma concentrations of IL-18 have been previously associated with the occurrence of MI and acute fatal coronary events.

The mechanism by which IL-18 genotype affects the occurrence of SCD remains unclear. It is possible that this genetic effect is due to an altered predisposition to traditional risk factors. We decided to examine whether traditional risk factors interact with IL-18 thus possibly impacting the occurrence of autopsy-verified CAD and SCD.

Methods

Subjects

Current study consists of two separate studies: The Helsinki Sudden Death Study (HSDS) and the Tampere Vascular Study (TVS). HSDS is an autopsy study comprising two independent autopsy series collected in 1981–1982 (A series, n = 400), and 1991–1992 (B series, n = 300). The autopsies were performed on Finnish Caucasian men (mean age of 53 years, range 33–70 years). All the men were subjected to a medicolegal autopsy because of their unexpected and often unwitnessed sudden death occurring outside a hospital. A medicolegal autopsy was not performed if the deceased had a previous clinical diagnosis of CAD complicated by, for example, severe chronic heart failure. All medicolegal autopsies were performed according to the same protocol at the Department of Forensic Medicine at the University of Helsinki, and the study was approved by the Ethics Committees of the Department and the University. The details of this study have been described in detail earlier. The TVS material consists of arterial samples of atherosclerotic lesions (types V–VI) from carotid arteries (n = 9), femoral arteries (n = 4), and the aortas (n = 7) of 20 patients subjected to vascular surgical procedures and healthy control samples of the left interior thoracic artery and the left internal mammary artery of patients (n = 6) undergoing coronary artery bypass surgery in Divisions of Vascular and Cardiothoracic Surgery, Tampere University Hospital. Genome wide expression analysis (GWEA) was performed on all the samples. Of the patients, 15 were GG homozygotes, 9 GC heterozygotes, and 1 was a CC homozygote. The patients were divided into two groups: GG homozygotes and C allele carriers. The expression levels of IL-18 receptor α (IL-18Rα), IFN-γ, and IL-12 have been previously measured using real-time polymerase chain reaction (PCR) for a separate publication, and these measurements were incorporated into the data to obtain more accurate results.

Genotyping of the IL-18 polymorphism

The DNA samples were genotyped using the S’ nuclease assay for allelic discrimination with the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Genotyping of the IL-18 SNP (rs187238) was successful for 663 (94.7%) of the 700 tissue samples in the HSDS and 25 (96.2%) of the 26 tissue samples in the TVS. The method of genotyping by PCR has been described in detail earlier. Random duplicates were used as a quality control.

Post mortem verification and classification of varying causes underlying sudden cardiac death

Of all the SCDs in the present material, 220 (80%) were caused by CHD. Most frequently, the cause of death was acute or old MI (n = 154). Of these deaths, 101 were caused by an acute MI, 53 were associated with the scar of a prior MI and were most probably due to arrhythmias, and 64 men suffered a CHD-related SCD with no MI aetiology. Myocardial infarction was verified by means of a macroscopic and histological examination of the myocardium. The presence of a coronary thrombus was recorded during autopsy, when coronary arteries were opened longitudinally.

Autopsy measurement of coronary stenosis and atherosclerosis

At autopsy, a silicon rubber cast was made from the three main epicardial coronary arteries (the left anterior descending, left circumflex, and right coronary artery). The degree of coronary stenosis was determined from these rubber models. The cut-off value for the classification of CAD was over 50% stenosis in any part of one or several main coronary arteries. In order to analyse the areas of different types of atherosclerotic lesions and overall atherosclerosis, the coronary arteries were fixed in 10% buffered formalin and stained for fat by the Sudan IV staining method. The methods of these measurements have been described previously.

Collection of risk factor data in the HSDS

A close friend, spouse, or relative of the deceased was interviewed by means of a questionnaire to obtain risk factor data. Complete interview data on risk factors were available in 402 (60.6%) of the 663 cases whose IL-18 genotype was successfully determined. Risk factor data included the following variables: hypertensive (yes/no), diabetic (yes/no), smoker (yes/no) (smokers and ex-smokers were combined into the category of smokers for the statistical analysis), daily alcohol consumption, and body mass index (BMI). Body mass index...
was calculated from the autopsy data. The victim was considered hypertensive if his hypertension had been diagnosed clinically by a physician and/or he was medically treated for hypertension, or if it was known that hypertensive blood pressure (BP) values had been measured from the subject prior to his death.

RNA isolation and genome wide expression analysis

The fresh tissue samples (n = 26) were immediately soaked to RNA-Later solution (Ambion Inc., Austin, TX, USA) and total-RNA was isolated with Trizol reagent (Invitrogen, Carlsbad, CA, USA) and RNAEasy Kit (Qiagen, Valencia, CA, USA). The GWEA Microarray experiments were performed by using Sentrix® Human-8 Expression BeadChips analysing over 23,000 known genes and gene candidates (Illumina, San Diego, CA, USA) according to the instructions given by the manufacturer. BeadChips were scanned with Illumina BeadArray Reader. The method has been more accurately described in our earlier work. The accuracy of Illumina Sentrix® Human-8 Expression BeadChips microarray methodology to measure the gene expression was verified by real-time quantitative TaqMan® PCR through quantifying the expression of 20 genes with both methods.

Statistical analyses

Because of the small number of CC homozygotes (n = 43, 6.5%) in the HSDS, they were combined with the GC heterozygotes to form a group of C allele carriers for statistical analyses. However, the analyses were also repeated without pooling the genotypes.

In order to compare the characteristics between genotype groups, we used Pearson’s χ²-test for categorical variables and the Mann–Whitney U test for continuous variables. Binary logistic regression analysis was used to calculate the IL-18 genotype-by-risk-factor interactions. Analyses for each genotype group by risk factor interaction were carried out with and without adjustment for autopsy data (BMI and age) and interview data (alcohol consumption, smoking, diabetes, and hypertension). Covariates were included in the model in a stepwise manner. Only statistically significant (P < 0.05) covariates were accepted in the final adjusted model. If the interaction between IL-18 genotype group and a risk factor was found significant, the effect of the risk factor on the occurrence of SCD was studied separately using unadjusted and adjusted binary logistic regression analysis (stepwise procedure for accepting significant covariates) stratifying the population by IL-18 genotypes.

The control group consisted of men who had died of other diseases and men who had died of unnatural causes. This was included because the IL-18 genotype group did not associate with the rate of deaths caused by non-cardiac-related diseases or unnatural causes.

In the TVS material, the expression levels of IL-18 mRNA and IFN-γ mRNA were compared over IL-18 genotype groups, and patients with or without hypertension using adjusted analysis of variance (ANOVA). All ANOVAs were adjusted with the mRNA expression of surface structures expressed by antigen presenting cells (APC) (CD80 and CD86 transcript variant 1 (v1) and CD86 transcript variant 2 (v2)) and by T-cells (CD4, CD28, and CTLA-4). Macrophages which act also as APCs are major producers of IL-18 and T cells are major producers of IFN-γ. Only significant covariates were selected into the model by a backward elimination procedure. This allowed us to compare the expression of IL-18 and IFN-γ between samples with different inflammatory background, and it was necessary because the current material consist of atherosclerotic samples from different vascular beds with variation in inflammatory background. The expression of the mRNA of these factors is subjected to inflammatory stimuli. Thus, they do not represent directly the quantity of the T-cells or APCs in the samples. However, at the same time, they provide some adjustment on the inflammatory activity within the samples. All analyses were repeated with log-transformation of the continuous variables, but this did not improve the predictive value of the analyses (measured by pseudo R²-value), and thus the results of the analyses performed with crude values are reported. The study population was divided into two groups by median systolic arterial pressure (140 mmHg) in order to study the effect of hypertension on the dependent variables.

In both studies, the significance P-value < 0.05 was considered statistically significant. Values of significance on all non-parametric tests are presented as asymptotic and two-tailed. The computations were carried out with SPSS for Windows software (Version 14.0, SPSS Inc., TX, USA).

Results

Characteristics of the study subjects in the HSDS

The mean age of the study population at the time of death was 53.2 ± 9.5 years (range 33–70 years). The characteristics of the study population are presented in Table 1. The genotype frequencies in the order of GG-GC-CC were: 359 (54.1%), 261 (39.4%), and 43 (6.5%). The allelic frequencies were 0.262 for the C allele and 0.738 for the G allele. The genotype distribution was in accordance with the Hardy–Weinberg equilibrium (P = 0.89 by χ²-test). The genotype groups did not have any significant associations with any common CAD risk factors or with the occurrence of CAD, nor were there any significant differences (observed) between subjects with or without interview data available (data not shown).

The interactions between risk factors and IL-18 gene polymorphism on the risk of sudden cardiac death and coronary artery disease

According to unadjusted binary logistic regression analysis, IL-18 genotype group interacted significantly with hypertension (crude P = 0.009 and adjusted P = 0.011) as regards to the effect on the risk of SCD. After applying the Bonferroni correction, the interaction remained significant (crude P = 0.045 and adjusted P = 0.055). When the IL-18 genotype information was entered into the analysis without pooling CG heterozygotes and CC homozygotes, the result remained significant (crude P = 0.022 and adjusted P = 0.029). Interactions with daily alcohol consumption (P = 0.600), BMI (P = 0.075), smoking (P = 0.998), or diabetes (P = 0.943) were not statistically significant.

Similarly, in unadjusted binary regression analysis of the occurrence of CAD, there was a statistically significant interaction between IL-18 genotype group and hypertension (P = 0.020), which remained significant after an adjustment for other risk factors (P = 0.026). After Bonferroni correction, the interaction was no longer significant (crude P = 0.100 and adjusted P = 0.130). Interactions with daily alcohol consumption (P = 0.701), BMI (P = 0.833), smoking (P = 0.805), and diabetes (P = 0.316) were not statistically significant.
The effect of hypertension on the risk of sudden cardiac death and coronary artery disease among IL-18 genotypes

Because of the statistically significant genotype-group-by-hypertension interaction, we divided the study population according to IL-18 genotype. Among GG homozygotes and C allele carriers, hypertensive men were statistically significantly older and had higher BMI compared with normotensive men (Table 2). The same associations were also observed separately among the 226 GC heterozygotes (P = 0.023 for age and P = 0.001 for BMI), but no longer among the 43 CC homozygous men (n = 0.927 for age and P = 0.118 for BMI). No other statistically significant differences were observed between hypertensive and normotensive men in either genotype group. Among SCD victims, 23.9% (n = 55) had suffered from hypertension. In the control group, the corresponding number was 10.4% (n = 43).

Hypertension was a major risk factor of SCD and CAD among GG homozygotes, but not among combined group of C allele carriers or among GC heterozygotes or CC homozygotes, when the genotypes were analysed separately (Table 3). Among GG homozygotes, hypertension associated significantly with SCD due to CHD (adjusted OR 3.75, P = 0.0005; Figure 1), SCD due to old or acute MIs (SCD due to arrhythmias caused by an old MI scar and/or AMI) (adjusted OR 4.56, P = 0.0002), fatal acute MI (adjusted OR 4.69, P = 0.0004), and with CAD (adjusted OR 3.08, P = 0.0027; Figure 1). Among the CC homozygotes, the effect of hypertension on the risk of SCD and CAD was evaluated by Fisher’s exact χ²-test because only five men had suffered from hypertension, one of them had suffered SCD, and only two had CAD. According to the χ²-test, hypertension was not associated with SCD due to CHD (P = 0.632), SCD due to old or acute MI (P = 1.00), fatal acute MI (P = 1.00), or with CAD (P = 1.00).

The effect of hypertension on the different atherosclerotic plaque areas in the HSDS

In order to study how hypertension affects the atherosclerotic plaque formation and composition among IL-18 genotype...
groups, we focused on the control group. This was done to avoid the evident selection bias presented by the significantly different mortality to CHD-related SCD among different IL-18 genotype groups. Among GG homozygotes, according to ANOVA adjusted with autopsy data (BMI and age), the coronary arteries of hypertensive men were more afflicted by overall atherosclerosis.

Table 3 The effect of hypertension on the risk of sudden cardiac death and coronary artery disease among GG homozygotes, C allele carriers and GC heterozygotes of the IL-18 – 137 (G/C) polymorphism

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted OR (95% CI)</th>
<th>P-Value</th>
<th>Adjusted OR (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GG homozygotes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCD by CHD</td>
<td>4.21 (2.28–7.78)</td>
<td>&lt;0.00001</td>
<td>3.75 (1.78–7.91)</td>
<td>0.0005</td>
</tr>
<tr>
<td>SCD with MI aetiology</td>
<td>5.02 (2.63–9.57)</td>
<td>&lt;0.000001</td>
<td>4.56 (2.05–10.11)</td>
<td>0.0002</td>
</tr>
<tr>
<td>SCD by fatal acute MI</td>
<td>4.75 (2.33–9.68)</td>
<td>0.00002</td>
<td>4.69 (2.01–10.95)</td>
<td>0.0004</td>
</tr>
<tr>
<td>CAD</td>
<td>4.42 (2.40–8.13)</td>
<td>&lt;0.000002</td>
<td>3.08 (1.48–6.41)</td>
<td>0.0027</td>
</tr>
<tr>
<td><strong>CG heterozygotes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCD by CHD</td>
<td>0.35 (0.04–3.45)</td>
<td>0.369</td>
<td>0.75 (0.28–2.00)</td>
<td>0.565</td>
</tr>
<tr>
<td>SCD with MI aetiology</td>
<td>1.69 (0.71–3.99)</td>
<td>0.233</td>
<td>1.06 (0.36–3.10)</td>
<td>0.922</td>
</tr>
<tr>
<td>SCD by fatal acute MI</td>
<td>1.88 (0.68–5.14)</td>
<td>0.222</td>
<td>1.30 (0.40–4.22)</td>
<td>0.661</td>
</tr>
<tr>
<td>CAD</td>
<td>1.54 (0.73–3.23)</td>
<td>0.259</td>
<td>0.83 (0.32–2.11)</td>
<td>0.687</td>
</tr>
<tr>
<td><strong>C allele carriers</strong></td>
<td></td>
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<tr>
<td>SCD by CHD</td>
<td>1.19 (0.57–2.46)</td>
<td>0.643</td>
<td>0.78 (0.32–1.91)</td>
<td>0.583</td>
</tr>
<tr>
<td>SCD with MI aetiology</td>
<td>1.43 (0.64–3.18)</td>
<td>0.390</td>
<td>0.88 (0.32–2.40)</td>
<td>0.805</td>
</tr>
<tr>
<td>SCD by fatal acute MI</td>
<td>1.66 (0.66–4.20)</td>
<td>0.282</td>
<td>1.14 (0.39–3.37)</td>
<td>0.814</td>
</tr>
<tr>
<td>CAD</td>
<td>1.48 (0.73–2.97)</td>
<td>0.276</td>
<td>0.78 (0.32–1.89)</td>
<td>0.581</td>
</tr>
</tbody>
</table>

P-values have been derived with regression analysis. Autopsy data (BMI and age) and interview data (alcohol consumption, smoking, diabetes, and hypertension) were used to adjust the analyses. Covariates were included in the model in a stepwise manner. Only statistically significant (P < 0.05) covariates were accepted in the final adjusted model.

SCD, sudden cardiac death; CHD, coronary heart disease; MI, myocardial infarction; CAD, coronary artery disease.

Figure 1 The effect of hypertension on the risk of sudden cardiac death (SCD) and coronary artery disease (CAD) among the GG homozygotes and C allele carriers of the IL-18 gene – 137G/C polymorphism. Odds ratios (OR) are derived by logistic regression adjusted with traditional risk factors for CAD.
(measured as the relative surface area covered by atherosclerotic lesions) [11.71% (SE 1.72) vs. 6.18% (SE 0.48), P = 0.002] when compared with normotensive men. Hypertensive men also had larger relative areas of fatty streaks [7.14% (SE 1.34) vs. 4.21% (SE 0.40), P = 0.026] and fibrotic plaques [4.57% (SE 0.66) vs. 1.97% (SE 0.21), P < 0.001]. According to adjusted regression analysis, hypertension was also a significant risk factor for the occurrence of complicated plaques (OR 8.38 with 95% CI 2.39–29.33, P < 0.001, covariates: BMI and age).

Among C allele carriers, hypertension was not associated with the overall atherosclerotic burden [9.34% (SE 1.08) vs. 8.38% (SE 0.72), P = 0.701], relative surface areas of fatty streaks [6.80% (SE 1.03) vs. 5.17% (SE 0.50), P = 0.306], surface areas of fibrotic plaques [2.53% (SE 0.62) vs. 3.21% (SE 0.43), P = 0.268], or with the occurrence of complicated plaques (OR 2.07 with 95% CI 0.73–5.89, P = 0.174). In addition, when the groups of GC heterozygotes and CC homozygotes were analysed separately, hypertension did not associate significantly with the plaque areas or with the occurrence of complicated plaques.

The effect of IL-18 genotype and hypertension on the expression of IL-18 and IFN-γ in atherosclerotic arterial samples (TVS)

According to adjusted ANOVA, the effect of hypertension on the expressions of IL-18 mRNA and IFN-γ mRNA were modulated by the IL-18 genotype [P = 0.030 (IL-18) and P = 0.004 (IFN-γ) for the interactions]. Among GG homozygotes, hypertension did not associate with the expression level of intracellular precursor IL-18 mRNA (0.89-fold increase, P = 0.217), whereas among C allele carriers hypertension augmented the expression of intracellular precursor IL-18 mRNA (1.31-fold increase, P = 0.001). Almost inversely, among GG homozygotes hypertension associated with a higher expression level of pro-atherosclerotic IFN-γ (1.58-fold increase, P = 0.006), whereas among C allele carriers hypertension seemed to associate with a lower expression level of IFN-γ (0.58-fold increase, P = 0.047).

In the atherosclerotic samples obtained from GG homozygotes, the expression levels of the intracellular precursor IL-18 mRNA were lower (0.68-fold increase, P < 0.001), but the expression levels of IFN-γ mRNA were higher (1.85-fold increase, P < 0.001) when compared with the atherosclerotic samples of the C allele carriers. Furthermore, according to unadjusted Mann–Whitney U test, the expression levels of the IL-18 mRNA seemed to be significantly higher in the healthy control samples when compared with the atherosclerotic samples (1.33-fold increase, P = 0.052).

Significant covariates associating with the expression of IL-18 mRNA were: CD80 (P = 0.006), CD86v1 (P = 0.012), CD4 (P = 0.013), CD86v2 (P = 0.010), Caspase-1 variant α (P = 0.023), and Caspase-1 variant ε (P = 0.009). The expression levels of Caspase-1 (Casp-1) variants were also introduced to this model because Casp-1 cleaves the IL-18 precursor protein into biologically active IL-18 resulting in the secretion of mature IL-18 protein. This intrinsic processing likely affects the amount of intracellular IL-18 precursor mRNA, which has a stable mRNA structure and is constitutively and intracellularly stored.35

Significant covariates associating with the expression of IFN-γ mRNA were: CD80 (P < 0.001), CD86v1 (P < 0.001), CD4 (P = 0.014), CTLA-4 (P = 0.010), IL-12 (P = 0.004), and IL-18Rα (P = 0.003). The expression levels of IL-12 and IL-18Rα were also included into this model because IL-12 in synergy with IL-18 augments the production of IFN-γ and IL-18Rα plays an important role in IL-18 signalling.35 The expression of IL-18 binding protein, Casp-1vα, and Casp-1vε was also added to the analysis, but these covariates were not significant in the model and thus were not used for further adjustment.

Discussion

According to the present study, the IL-18 genotype significantly modifies the association of arterial hypertension with the occurrence of SCD due to CHD. We found that arterial hypertension was a major risk factor for SCD and severe CAD among GG homozygotes, but not among C allele carriers of the −137 (G/C) polymorphism (rs187238). When we studied the risk of SCD due to MI (SCD due to arrhythmias caused by an old MI scar and/or AMI) or the risk of fatal acute MI, the results were even more pronounced despite the lower sample sizes of the subgroups. The results of the autopsy data of the independent control group showed that hypertension associates significantly with development of overall coronary atherosclerosis as well as with the occurrence of complicated plaques among GG homozygotes, but not among C allele carriers. To confirm and investigate further this interaction, we performed detailed analysis of gene expression in atherosclerotic samples from live patients of the TV study. The results showed that the effect of hypertension on the expressions of IL-18 mRNA and IFN-γ mRNA is modulated by the IL-18 genotype.

Previously, it has been shown that circulating IL-18 levels are higher among patients with unstable angina pectoris and among MI victims.24,26,36 Among CAD patients, higher IL-18 levels are associated with a higher risk of a future fatal coronary event.25 Higher IL-18 levels have also been found to predict future coronary events among healthy middle-aged men.37 The studied promoter G-to-C polymorphism of the IL-18 gene has been proven functional with the C allele, associating with lower transcriptional activity of the IL-18 gene and thus resulting in an attenuated production of IL-18.12–14 Supporting these previous results, recent haplotype studies have shown that the variation in the IL-18 gene associates with circulating IL-18 levels.16,38 The same studies also showed that a major haplotype which is the sole carrierg of the −137C allele associates with lower IL-18 levels and with cardiovascular mortality during a follow-up of 5.9 years. One other major haplotype, the only one carrying the G allele of the A-to-G polymorphism at position +183, was similarly associated with the same endpoints. Other haplotypes or SNPs of this gene were not found to associate with circulating IL-18 levels or cardiovascular mortality.16,38 Based on these previous results, it seems clear that IL-18 gene variation affects cardiovascular mortality. However, according to our new results, the
association between this polymorphism and CHD-related SCD seems more complex.

High BP values have previously been associated with high circulating IL-18 concentrations. Furthermore, circulating IL-18 values along with greater intima media thickness values could be treated effectively by lowering morning BP peaks with a 12 month treatment with the unselective β-blocker carvedilol. However, this does not explain why hypertension seems to associate with the risk for SCD only among IL-18 GG homozygotes.

In the present study, we found that IL-18 genotype significantly modulates the effect of hypertension on the expression of IL-18 mRNA and IFN-γ mRNA in atherosclerotic tissue samples obtained from live patients. It is especially noteworthy that among GG homozygotes, hypertension associated with higher expression level of the pro-atherosclerotic IFN-γ. In the group of C allele carriers, hypertension actually associated with a higher expression level of the intracellular IL-18 mRNA. Previously, it has been shown that β1-adrenergic receptor stimulation activates both basal and inducible IL-18 promoter activity in endothelial cells leading to upregulation of IL-18 mRNA expression, and more stable IL-18 mRNA. Also, it has been shown in an in vitro study that aldosterone in cardiomyocytes, through the production of angiotensin II and endothelin-1, increase IL-18 mRNA and protein expression. Unfortunately, it is not possible to deduct more conclusions from our expression analysis results because the regulation of the different genetic pathways is highly complex, and thus more accurate and extensive data would be required.

Hypertension might also have a more general effect on the occurrence of SCD and formation of atherosclerosis. The overall pro-inflammatory effects of higher BP on the vessel wall may lead to increased production of mature IL-18 by endothelial cells and macrophages. Also the C allele carriers might be better protected against this because of their impaired production of IL-18. Furthermore, Sahar et al. have shown that pre-treatment of vascular smooth muscle cells with angiotensin II increases the expression of the ligand-binding subunit of IL-18Rα of the IL-18 receptor, and thus leads to IL-18-mediated induction of the transcription factor nuclear factor-κB (pathway leading to the expression of several pro-inflammatory cytokines and chemokines and the production of pro-inflammatory cytokines such as IL-6 and IL-8).

A major limitation of our study is the fact that we do not have the genotype data concerning the +183A/G polymorphism (rs5744292) which has also been found to significantly affect circulating IL-18 levels. However, as the haplootype carrying the +183G allele is also associated with attenuated production of IL-18, it only emphasizes our results because these two SNPs do not co-exist in a same haplotype. Therefore, among GG homozygotes of the −137G/C polymorphism, there are more G allele carriers of the +183A/G polymorphism than among the C allele carriers. Furthermore, we had no data on the serum IL-18, cholesterol, and other lipid values of the deceased men. In addition, we were unable to obtain complete interview data on all subjects, and thus we were unable to analyse the possible effect of pre-mortem medication on the IL-18 genotype-by-hypertension interaction.

The information on hypertension in the present study was based on interview data which cannot be considered a valid data based on recorded clinical measurements. However, previous studies have shown that the validity of interview data concerning hypertension is high, and moreover, it is more likely for hypertensive men to have been misdiagnosed as normotensive, than normotensive men as hypertensive. This suggests that the reliability of our results would not be significantly weakened by this limitation. More likely, our results would be more pronounced if the accuracy of the diagnosis would be better. Other limitations concerning the study population have been discussed earlier. The strength of the present study is the fact that it comprises almost all SCDs which occurred in the Helsinki region during the time of the collection of the autopsy series, making our study population a valid representative of a population at the risk of SCD.

In conclusion, our results suggest that the effect of hypertension on the development of CHD leading to an untimely SCD and the development of overall coronary atherosclerosis as well as the occurrence of complicated plaques is modulated by the IL-18 gene promoter region −137G/C polymorphism. Hypertension seems to be a major risk factor for these endpoints among GG homozygous men, but not among C allele carriers.

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Conflict of interest: none declared.

References


