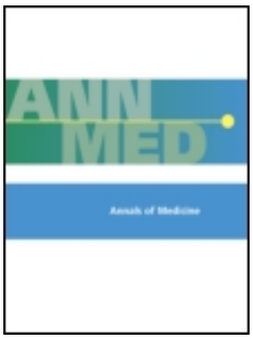

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ORIGINAL ARTICLE

Interleukin-18 gene polymorphism and markers of subclinical atherosclerosis. The Cardiovascular Risk in Young Finns Study

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Abstract

Background and aim. Interleukin-18 (IL-18) is a pro-atherosclerotic cytokine. We wanted to evaluate whether IL-18 gene polymorphism associates independently of risk factors, with early subclinical markers of atherosclerosis (intima-media thickness (IMT), coronary artery compliance (CAC), and flow-mediated dilatation (FMD)) in a population of young healthy Caucasian adults.

Methods. This study was based on the on-going Cardiovascular Risk in Young Finns Study consisting of 2260 young adults, mean age being 31.7 (range 24–39 years) (1247 women and 1013 men).

Results. Five studied tagSNPs formed six major haplotypes, which accounted for 99.9% of all variation of the IL-18 gene. According to adjusted analysis of variance, the IL-18 gene polymorphism did not associate with subclinical atherosclerosis in the whole study population. However, one major haplotype associated differently among men and women with IMT ($P = 0.011$). Male carriers of a major CCTgT haplotype ($n = 441$) seemed to have a lower IMT when compared to the non-carriers (−0.016 mm, 95% confidence interval (CI) −0.028 to −0.004, $P = 0.014$). Among women no significant associations were observed.

Conclusions. Among all study subjects, the polymorphism of the IL-18 gene is not associated with subclinical markers of atherosclerosis. However, among men one major IL-18 haplotype seemed to associate with substantially lower IMT values.

Key words: *Arterial elasticity, atherosclerosis, genetics, inflammation, intima-media thickness*

Introduction

Atherosclerosis is an inflammatory disease which progresses through decades (1,2). Interleukin-18 (IL-18), a pro-inflammatory and pro-atherosclerotic cytokine is produced mainly by monocytes and macrophages (3,4). It seems to play a crucial role in the development of more vulnerable atherosclerotic plaques (5,6) through inducing the production of interferon-γ (7).

In-vitro models have shown that the genetic variation of the IL-18 gene affects the monocyte's production of IL-18 (8–11). Likewise, the expression of IL-18 in humans is regulated by the genetic variability of the IL-18 gene, and according to several studies IL-18 gene polymorphism associates with circulating IL-18 levels (12–15).

Previously IL-18 levels have been shown to correlate with the extent of coronary atherosclerosis

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Key messages

- Interleukin-18 (IL-18) gene polymorphism has been previously associated with severe clinical end-points such as sudden cardiac death and the occurrence of myocardial infarctions.
- According to the results of the present haplotype study, the IL-18 gene polymorphism does not associate significantly with subclinical markers of atherosclerosis among a population of healthy young adults.
- However, among men one major haplotype seems to associate significantly with lower intima-media thickness of the carotid artery.

Abbreviations

ANOVA	analysis of variance
ApoA-1	apolipoprotein A-1
ApoB	apolipoprotein B
BMI	body mass index
BP	blood pressure
CAC	carotid artery compliance
CAD	coronary artery disease
CRP	C-reactive protein
FMD	flow-mediated dilatation
HDL	high-density lipoprotein
IL-18	interleukin-18
IMT	intima-media thickness
LD	Llow-density lipoprotein
MI	myocardial infarction
SCD	sudden cardiac death
SNP	single nucleotide polymorphism

among patients with previous myocardial infarction (MI) and with unstable angina (16,17) and also to predict the mortality in patients with coronary artery disease (CAD) (18,19). Genetic studies have revealed similar results: Polymorphism of the IL-18 gene is associated with cardiovascular mortality among CAD patients and MI risk among hypertensive patients and post-menopausal women (12,20).

Increased carotid artery intima-media thickness (IMT), elasticity, and flow-mediated dilatation (FMD) are early subclinical markers of atherosclerosis and predict future coronary events (21–23). Carotid artery compliance (CAC) depicts the ability of the arteries to expand under the influence of pulse pressure. Diminished arterial elasticity has been shown to be an independent predictor of cardiovascular events in high-risk individuals (24,25). FMD of the brachial artery quantifies the amount of vasodilatation in response to endothelial activation by an increase in local blood-flow (26). Even before anatomical evidence of atherosclerosis appears, FMD is impaired in young symptom-free subjects with risk factors for vascular disease (27). Carotid IMT has been shown to predict independently future vascular events especially among young subjects (21).

The increased circulating levels of IL-18 seem to be associated with greater carotid artery IMT (28,29). Whether this association is independent of traditional risk factors is still unclear (28,29). To the best of our knowledge the relations of FMD and CAC to IL-18 concentrations or IL-18 genotypes have not been previously studied.

Lately the reports have concentrated on studying the IL-18 levels/polymorphism and their association to the advanced atherosclerotic end-points, e.g. acute MI, CAD. Thus we wanted to study whether known IL-18 haplotypes or single-locus tagSNP polymorphisms affect, independently of risk factors, the early (subclinical) markers of atherosclerosis (IMT, CAC, and FMD) in a population of young healthy Caucasian adults.

Materials and methods

Subjects

The Cardiovascular Risk in Young Finns Study is an on-going prospective multicentre cohort study, which provided us with the study population of 2282 young adults. Details of the cohort have been published previously (30–32). The study began in 1980, and the 21-year follow-up was carried out in 2001. All of the data used in the present study were collected in the year 2001. Participants with type 1 diabetes were excluded from further analyses. The Ethical Review Committee of Turku University Hospital approved the research plan, and the study followed the tenets of the Declaration of Helsinki. Patients gave informed consent before entering to the study.

Clinical and biochemical characteristics

A standardized questionnaire was used to assess cardiovascular risk factors (smoking, alcohol consumption, geographical origin, and familial history of coronary heart disease). The classification of these variables has been described earlier in more detail (33). Study subjects' height and weight were used to calculate their body mass index (BMI = weight, kg/(height, m)^2), and their blood pressure (BP) was recorded. Fasting venous blood samples were used to determine C-reactive protein (CRP), insulin, glucose, serum lipids, apolipoprotein A-1 (ApoA-1), apolipoprotein B (ApoB), and homocysteine concentrations. Total cholesterol, high-density lipoprotein (HDL), and triglycerides were determined enzymatically.

Low-density lipoprotein (LDL) was calculated by the Friedewald formula. Standardized methods were used in all determinations (31,32). The information of geographical origin was included as a covariate because the population originating from eastern Finland is genetically more predisposed to the development of atherosclerosis than is the population of western Finland (34).

DNA isolation and genotyping of the IL-18 polymorphism

Genomic DNA was isolated from peripheral blood leukocytes by using QIAamp®DNA Blood Minikit and automated Biorobot M48 (Qiagen, Hilden, Germany) extraction. The five IL-18 single nucleotide polymorphisms (SNPs) (rs1946519, rs360717, rs549908, rs5744292, rs4937100) and haplotypes were genotyped by using the 5′ nuclease assay for allelic discrimination and fluorogenic TaqMan MGB probes with the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Forster City, CA, USA) (35). The nucleotide sequences of the primers and fluorogenic allele-specific oligonucleotide probes used in polymerase chain reaction (PCR) were deduced from published sequences in the Gene Bank database. They were chosen and synthesized in co-operation with Applied Biosystems. PCR reaction containing genomic DNA, 2 × TaqMan Universal PCR Master Mix, 900 nM of each primer, and 200 nM of each probe was performed in 384well plates according to standard protocol in a total volume of 5 μL. Water controls and random duplicates were used as a quality control.

Measurements of subclinical markers of atherosclerosis (IMT, CAC, and FMD)

Carotid artery IMT was measured by ultrasound, and for determining CAC the concomitant brachial blood pressure was also monitored. The brachial artery FMD was assessed by measuring the left brachial artery diameter both at rest and during reactive hyperemia. Ultrasound studies were performed using Sequia512 mainframes (Acuson, Mountain View, CA, USA) with a 13.0 MHz linear array transducer. The procedures have been earlier discussed in more detail (32).

To determine the intra-individual reproducibility of the measurements, the ultrasound measurements were replicated for a small random sample of the participants ($n = 57$, 2.5%) 3 months after the initial visit. The between-visit coefficient of variation was 6.4% for IMT, 16.3% for CAC, and 26% for FMD measurements.

Statistical analyses

The statistical analysis was performed using the SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA). In order to study the possible association between IL-18 gene polymorphism and the subclinical markers of atherosclerosis, we applied adjusted analysis of variance (ANOVA). Only significant covariates were accepted into the models (the selection criteria and procedure have been previously described in more detail) (33). First we studied the possible factor-by-sex interactions. If the interaction between the haplotype or SNP and sex was found to be significant we proceeded to analyse the effect of the haplotype or SNP among men and women separately, and the results from the level of the whole study population were not interpreted. To study the effect of haplotypes, we divided the population into carriers and non-carriers. The effects of individual genotypes were studied without pooling the genotypes. Previously pregnancy has been associated with changes in serum values of IL-18, and this tendency has been found to be modulated by IL-18 genotype (36,37). For this, the analyses were performed before and after the exclusion of the 61 women who were pregnant during the time of the study. However, the results remained almost unchanged, and thus we only present the results obtained from the whole study population.

ANOVA was used to evaluate the possible association between risk factors and the polymorphism of the IL-18 gene. Variables with skewed distributions (insulin, glucose, and CRP) were $\log_{10}$-transformed for the analyses. Categorical variables and Hardy-Weinberg equilibrium were tested using the chi-square test.

A value of $P < 0.05$ was considered statistically significant. In order to correct for multiple testing we applied the Li and Ji method (38). This method is the improved version of the method originally introduced by Nyholt et al. for adjusting for multiple testing in multi-locus analyses (39). This correction method was used because of the high linkage disequilibrium between the SNPs. Frequencies of the most common haplotypes and the most probable haplotypes for each study subject were determined using the PHASE program (Version 2.0.2) (40).

One of the IL-18 genotype distributions (533T>C, rs4937100) was not in Hardy-Weinberg equilibrium in our study population ($P < 0.001$). Nevertheless, we included it into our haplotype reconstruction in order to verify whether our haplotype frequencies would be in line with those observed in previous studies (12). This SNP was responsible for dividing one common haplotype (haplotype aCTA with a frequency of 0.173) into

two smaller haplotypes (aCTAT, frequency 0.132; and aCTAc, frequency 0.041) (see Table III below). Because the genotype distributions of the 533T>C SNP was not in Hardy-Weinberg equilibrium, this SNP was excluded from further analyses, and only the effect of the combined aCTA haplotype was studied.

Results

General characteristics

The mean age of the study population was 31.7 ± 5.0 (SD) years, and there were more women than men (n = 1247 versus n = 1013, respectively). The average BMI of the study population was 24.9 ± 4.2 SD. Other general characteristics are presented in Table I. Genotyping was successfully performed in 92.6%–99.6% of the cases depending on the genotype. Genotype distributions are presented in Table II.

Haplotype analyses

The five studied IL-18 SNPs (rs1946519, rs360717, rs549908, rs5744292, rs4937100) formed six major haplotypes: CCTAc, atgAT, CCTgT, aCTAT, CCgAT, and aCTAc. The haplotype frequencies, presented in Table III, were in line with the results of the earlier haplotype study by Tiret et al. (12). These haplotypes accounted for 99.9% of all variation in the IL-18 gene. The CCTgT haplotype was the only haplotype carrying the G allele of the 415A>G polymorphism (rs5744292), and the atgAT haplotype was the only one to carry the T allele of the 127C>T polymorphism (rs360717).

IL-18 gene polymorphism and cardiovascular risk factors

None of the studied SNPs (Table II) or haplotypes (Table III) of the IL-18 gene associated with the clinical or biochemical risk factors of atherosclerosis measured in our study (variables in Table I) after correcting the analysis for multiple testing. Some significant differences were observed between the populations originating from eastern and western Finland before correcting for the number of tests performed within the group of risk factors. The frequencies of the minor alleles of the +35 (T/g) SNP (P = 0.005) and +127 (C/t) SNP (P = 0.040) were higher among the population with eastern Finnish origin when comparing to the population originating from western Finland.

IL-18 polymorphisms and markers of subclinical atherosclerosis

According to ANOVA adjusted for sex, BMI, age, smoking, and geographical origin, the CCTgT haplotype associated with carotid artery IMT significantly differently among men and women (P = 0.011 after correcting for multiple testing). Among men, the carriers of the CCTgT haplotype had a significantly lower IMT when compared to the non-carriers (adjusted difference in means: –0.016 mm, 95% CI –0.028 to –0.004, P = 0.014 after correcting for multiple testing). The adjusted mean values by haplotype groups were: 0.601 mm (standard error (SE) of 0.004 mm) for non-carriers, 0.585 mm (SE 0.005 mm) for heterozygous carriers, and 0.596 mm (SE 0.014 mm) for homozygous carriers of the CCTgT haplotype.

Table I. Characteristics of the study population.

Variable	All (n = 2260)	Male (n = 1013)	Female (n = 1247)	P-value[a]
Age (years)	31.7 ± 5.0	31.7 ± 5.0	31.7 ± 5.0	0.933
Body mass index (kg/m^2)	24.91 ± 4.16	25.69 ± 3.79	24.27 ± 4.33	<0.001
Ever smokers, yes (%)[b]	959 (42.4)	493 (48.7)	466 (37.4)	<0.001
Systolic BP (mmHg)	122.0 ± 14.4	129.2 ± 13.5	116.2 ± 12.4	<0.001
Diastolic BP (mmHg)	73.16 ± 9.0	74.99 ± 9.1	71.67 ± 8.7	<0.001
Cholesterol (mmol/L)	5.16 ± 0.98	5.26 ± 1.03	5.09 ± 0.93	<0.001
HDL cholesterol (mmol/L)	1.29 ± 0.32	1.16 ± 0.28	1.40 ± 0.31	<0.001
LDL cholesterol (mmol/L)	3.27 ± 0.85	3.42 ± 0.92	3.16 ± 0.78	<0.001
Triglycerides (mmol/L)	1.34 ± 0.85	1.53 ± 0.99	1.18 ± 0.68	<0.001
Apolipoprotein A-l (g/L)	1.50 ± 0.26	1.40 ± 0.21	1.57 ± 0.27	<0.001
Apolipoprotein B (g/dL)	1.06 ± 0.26	1.13 ± 0.27	1.00 ± 0.24	<0.001
Insulin (mU/L)	7.63 ± 5.21	7.52 ± 5.19	7.72 ± 5.22	0.382
Glucose (mmol/L)	5.00 ± 0.45	5.15 ± 0.42	4.87 ± 0.44	<0.001
C-reactive protein (mg/L)	1.89 ± 3.85	1.46 ± 3.27	2.23 ± 4.23	<0.001
Homocysteine (μmol/L)	9.82 ± 3.81	10.86 ± 4.18	8.97 ± 3.24	<0.001

Values of continuous variables are expressed as mean ± standard deviation.
[a]Significance calculated using one-way ANOVA for continuous variables and Pearson's chi-square test for categorical variables.
[b]This category includes daily smokers, ex-daily smokers, and occasional smokers.
BP = blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Table II. Interleukin-18 (IL-18) genotype distributions.

SNP, rs number	IL-18 genotype, *n* (%)		
	Major homozygote	Heterozygote	Minor homozygote
–656 (C/a), rs1946519	673 (30.0%)	1065 (47.1%)	506 (22.5%)
+127 (C/t), rs360717	1125 (50.8%)	899 (40.6%)	189 (8.5%)
+35 (T/g), rs549908	1062 (47.2%)	966 (42.9%)	223 (9.9%)
+415 (A/g), rs5744292	1204 (57.6%)	766 (36.6%)	121 (5.8%)
+533 (T/c)[a], rs4937100	1195 (53.4%)	704 (31.4%)	340 (15.2%)

[a]Genotype distribution not in Hardy-Weinberg equilibrium.
SNPs = single nucleotide polymorphisms.

The difference in IMT between heterozygous and homozygous male carriers of this haplotype was not significant (corrected $P = 1.00$). As the carriers of this CTCgT haplotype are the only ones carrying the minor g allele of the +415 C/g SNP (rs5744292), it is clear that the significant difference between the haplotype groups is caused by the minor g allele of the +415 C/g SNP. Among women no significant difference was observed (0.005 mm, 95% CI −0.004 to 0.014, corrected $P = 0.498$). None of the other haplotypes or SNPs associated significantly with IMT among the whole study population, and no other significant haplotype-by-sex interactions were seen. None of the haplotypes or studied SNPs associated significantly with CAC or brachial artery FMD in the whole study population, and we did not observe any significant haplotype-by-sex interactions. The exclusion of geographical origin from covariates left the results unchanged, and thus also the observed associations remained significant.

Discussion

According to the novel results of the present haplotype study, the variation of the IL-18 gene is not associated with subclinical markers of atherosclerosis among a population-based sample of healthy young adults. However, among men, carrying a major haplotype (CCTgT) of the IL-18 gene appears to associate with lower IMT independently of risk factors.

Thus far there are only contradictory results of the possible link between IL-18 and IMT. In 2005, Yamagami et al. reported that higher circulating IL-18 levels associated independently with greater carotid IMT among a population without a history of cardiovascular accidents (28). A later study by Chapman et al., conducted with a younger study population with lower overall atherosclerotic burden, was unable to replicate this finding (29). The fact that the present study lacks the data of circulating IL-18 levels is a weakness. However, according to prior evidence, the genetic variation of the IL-18 gene affects the circulating levels of IL-18 (12–15). In fact, the very same haplotype that associated significantly with lower IMT among men in our study was also found to associate with lower IL-18 levels and cardiovascular mortality in a large haplotype study by Tiret et al. (12). It is also a possibility that the local production of IL-18 within the atherosclerotic plaque might affect independently the overall development of the disease. Supporting the hypothesis, we have demonstrated that the mRNA expression of IL-18 in atherosclerotic plaques is dependent on IL-18 gene polymorphism (41). One IL-18 gene promoter region SNP (−137G/c, rs187238) was seen to associate with the expression of both IL-18 and Interferon γ (INF-γ) mRNA. This polymorphism, as well as one other SNP (rs360719), associating with the mRNA expression of IL-18 in peripheral blood monocytes, is in nearly complete concordance with

Table III. Interleukin-18 (IL-18) haplotypes.

	IL-18 SNPs						
	–656(C/a) rs1946519	+127(C/t) rs360717	+35(T/g) rs549908	+415(A/g) rs5744292	+533(T/c) rs4937100	Haplotype frequency	(SE)
Haplotype	C	C	T	A	c	0.270	(0.0005)
	a	t	g	A	T	0.288	(0.0003)
	C	C	T	g	T	0.244	(0.0003)
	a	C	T	A	T	0.132	(0.0005)
	C	C	g	A	T	0.024	(0.0001)
	a	C	T	A	c	0.041	(0.0004)
Minor allele frequency	0.462	0.288	0.313	0.240	0.312		

SNPs = single nucleotide polymorphisms.

one of the SNPs studied in the present work (rs360717) (8,12). The minor allele of the −137 G to C polymorphism (rs187238) is also linked with lower risk for sudden cardiac death (SCD) among men (41). Despite these findings, according to the present study, this polymorphism does not associate with the early stages of the disease.

In fact, most of the positive results linking IL-18 to atherosclerosis are derived from older and more risk factor-burdened patients (mean ages of patient groups ranging from 54 to 69 years) with more severe atherosclerotic disease end-points, e.g. acute MI, CAD, and SCD (12,16–20). Thus it seems that IL-18 has a significant role in the development of atherosclerosis in the later rather than in the early stage of the disease. Atherosclerosis also develops faster in men, and this could at least partly explain why the association between the CCTgT haplotype and IMT was seen among men but not among women. The male carriers of this CCTgT haplotype (also the only one carrying the g allele of the +415 C/g SNP) had 0.016 mm lower IMT compared to the male non-carriers. Clinically, this difference in IMT was greater in comparison to the effects of some more well known classical risk factors such as smoking (change of 0.011 mm), male sex (change of 0.010 mm), and BMI (0.011 mm change for 1 SD change in BMI (= 4.2 kg/m^2)) and systolic blood pressure (0.010 mm change of 1 SD change in BP (= 14.4 mmHg)) previously demonstrated in the same material (32). As men tend to suffer more frequently from cardiovascular diseases (42), and taking into consideration the size of the effect, it would be feasible to suggest that this finding could have clinical significance. However, this association should be replicated in independent populations before it can be considered significant.

In addition to the lack of measurements of the circulating IL-18 levels, another limitation of our study is that the blood pressure values that were used to derive the carotid artery elasticity were measured from the brachial artery. This limitation has been discussed earlier in more detail (43). An obvious strength of our study is the study design. Our results are based on a multicentre study with a randomly selected study population, and the risk factor data of this study are extensive and thus provide a good background for a genetic association study. Furthermore, with the five tagSNPs genotyped in the present study we were able to cover more than 99% of the variation of the IL-18 gene, and thus the associative data are comprehensive.

In summary, according to the present haplotype study the polymorphism of the IL-18 gene is not associated with the development of subclinical atherosclerosis (measured by intima-media thickness, carotid arterial elasticity, or with endothelial function measured by flow-mediated dilatation) among all young Caucasian adults. However, among men one major haplotype (CCTgT) seems to associate with substantially lower carotid artery IMT values.

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Declaration of interest: The authors declare no conflicts of interest. Jussi Hernesniemi and Arto Heikkilä contributed equally to this paper.

References

1. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med. 1999;340:115–26.
2. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352:1685–95.
3. Okamura H, Tsutsui H, Kashiwamura S, Yoshimoto T, Nakanishi K. Interleukin-18: a novel cytokine that augments both innate and acquired immunity. Adv Immunol. 1998; 70:281–312.
4. Mallat Z, Corbaz A, Scoazec A, Graber P, Alouani S, Esposito B, et al. Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. Circ Res. 2001;89:E41–5.
5. Mallat Z, Corbaz A, Scoazec A, Besnard S, Leseche G, Chvatchko Y, et al. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. Circulation. 2001;104:1598–603.
6. Mallat Z, Henry P, Fressonnet R, Alouani S, Scoazec A, Beaufils P, et al. Increased plasma concentrations of interleukin-18 in acute coronary syndromes. Heart. 2002;88: 467–9.
7. Nakamura K, Okamura H, Wada M, Nagata K, Tamura T. Endotoxin-induced serum factor that stimulates gamma interferon production. Infect Immun. 1989;57:590–5.
8. Sanchez E, Palomino-Morales RJ, Ortego-Centeno N, Jimenez-Alonso J, Gonzalez-Gay MA, Lopez-Nevot MA, et al. Identification of a new putative functional IL18 gene variant through an association study in systemic lupus erythematosus. Hum Mol Genet. 2009;18:3739–48.
9. Giedraitis V, He B, Huang WX, Hillert J. Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation. J Neuroimmunol. 2001;112:146–52.
10. Liang XH, Cheung W, Heng CK, Wang DY. Reduced transcriptional activity in individuals with IL-18 gene variants detected from functional but not association study. Biochem Biophys Res Commun. 2005;338:736–41.

11. Arimitsu J, Hirano T, Higa S, Kawai M, Naka T, Ogata A, et al. IL-18 gene polymorphisms affect IL-18 production capability by monocytes. Biochem Biophys Res Commun. 2006;342:1413–6.
12. Tiret L, Godefroy T, Lubos E, Nicaud V, Tregouet DA, Barbaux S, et al. Genetic analysis of the interleukin-18 system highlights the role of the interleukin-18 gene in cardiovascular disease. Circulation. 2005;112:643–50.
13. Barbaux S, Poirier O, Godefroy T, Kleinert H, Blankenberg S, Cambien F, et al. Differential haplotypic expression of the interleukin-18 gene. Eur J Hum Genet. 2007;15:856–63.
14. Thompson SR, McCaskie PA, Beilby JP, Hung J, Jennens M, Chapman C, et al. IL18 haplotypes are associated with serum IL-18 concentrations in a population-based study and a cohort of individuals with premature coronary heart disease. Clin Chem. 2007;53:2078–85.
15. Thompson SR, Novick D, Stock CJ, Sanders J, Brull D, Cooper J, et al. Free interleukin (IL)-18 levels, and the impact of IL18 and IL18BP genetic variation, in CHD patients and healthy men. Arterioscler Thromb Vasc Biol. 2007;27:2743–9.
16. Chen MC, Chen CJ, Yang CH, Wu CJ, Fang CY, Hsieh YK, et al. Interleukin-18: a strong predictor of the extent of coronary artery disease in patients with unstable angina. Heart Vessels. 2007;22:371–5.
17. Hulthe J, McPheat W, Samnegard A, Tornvall P, Hamsten A, Eriksson P. Plasma interleukin (IL)-18 concentrations is elevated in patients with previous myocardial infarction and related to severity of coronary atherosclerosis independently of C-reactive protein and IL-6. Atherosclerosis. 2006;188: 450–4.
18. Blankenberg S, Tiret L, Bickel C, Peetz D, Cambien F, Meyer J, et al. Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. Circulation. 2002;106:24–30.
19. Espinola-Klein C, Rupprecht HJ, Bickel C, Lackner K, Schnabel R, Munzel T, et al. Inflammation, atherosclerotic burden and cardiovascular prognosis. Atherosclerosis. 2007; 195:e126–34.
20. Bis JC, Heckbert SR, Smith NL, Reiner AP, Rice K, Lumley T, et al. Variation in inflammation-related genes and risk of incident nonfatal myocardial infarction or ischemic stroke. Atherosclerosis. 2008;198:166–73.
21. Lorenz MW, von Kegler S, Steinmetz H, Markus HS, Sitzer M. Carotid intima-media thickening indicates a higher vascular risk across a wide age range: prospective data from the Carotid Atherosclerosis Progression Study (CAPS). Stroke. 2006;37:87–92.
22. Simons PC, Algra A, Bots ML, Grobbee DE, van der Graaf Y. Common carotid intima-media thickness and arterial stiffness: indicators of cardiovascular risk in high-risk patients. The SMART Study (Second Manifestations of ARTerial disease). Circulation. 1999;100:951–7.
23. Schroeder S, Enderle MD, Ossen R, Meisner C, Baumbach A, Pfohl M, et al. Noninvasive determination of endothelium-mediated vasodilation as a screening test for coronary artery disease: pilot study to assess the predictive value in comparison with angina pectoris, exercise electrocardiography, and myocardial perfusion imaging. Am Heart J. 1999;138(4 Pt 1):731–9.
24. Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension. 2002;39:10–5.
25. Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. Hypertension. 1998;32:570–4.
26. Hijmering ML, Stroes ES, Pasterkamp G, Sierevogel M, Banga JD, Rabelink TJ. Variability of flow mediated dilation: consequences for clinical application. Atherosclerosis. 2001;157:369–73.
27. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet. 1992;340:1111–5.
28. Yamagami H, Kitagawa K, Hoshi T, Furukado S, Hougaku H, Nagai Y, et al. Associations of serum IL-18 levels with carotid intima-media thickness. Arterioscler Thromb Vasc Biol. 2005;25:1458–62.
29. Chapman CM, McQuillan BM, Beilby JP, Thompson PL, Hung J. Interleukin-18 levels are not associated with subclinical carotid atherosclerosis in a community population. The Perth Carotid Ultrasound Disease Assessment Study (CUDAS). Atherosclerosis. 2006;189:414–9.
30. Åkerblom HK, Viikari J, Uhari M, Räsänen L, Byckling T, Louhivuori K, et al. Atherosclerosis precursors in Finnish children and adolescents. I. General description of the cross-sectional study of 1980, and an account of the children's and families' state of health. Acta Paediatr Scand Suppl. 1985; 318:49–63.
31. Juonala M, Viikari JS, Hutri-Kähönen N, Pietikäinen M, Jokinen E, Taittonen L, et al. The 21-year follow-up of the Cardiovascular Risk in Young Finns Study: risk factor levels, secular trends and east-west difference. J Intern Med. 2004;255:457–68.
32. Raitakari OT, Juonala M, Kähönen M, Taittonen L, Laitinen T, Mäki-Torkko N, et al. Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. JAMA. 2003;290:2277–83.
33. Hernesniemi JA, Raitakari OT, Kähönen M, Juonala M, Hutri-Kähönen N, Marniemi J, et al. Toll-like receptor 4 gene (Asp299Gly) polymorphism associates with carotid artery elasticity. The cardiovascular risk in young Finns study. Atherosclerosis. 2008;198:152–9.
34. Juonala M, Viikari JS, Kähönen M, Taittonen L, Rönnemaa T, Laitinen T, et al. Geographic origin as a determinant of carotid artery intima-media thickness and brachial artery flow-mediated dilation: the Cardiovascular Risk in Young Finns study. Arterioscler Thromb Vasc Biol. 2005;25:392–8.
35. Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genet Anal. 1999;14:143–9.
36. Ida A, Tsuji Y, Muranaka J, Kanazawa R, Nakata Y, Adachi S, et al. IL-18 in pregnancy; the elevation of IL-18 in maternal peripheral blood during labour and complicated pregnancies. J Reprod Immunol. 2000;47:65–74.
37. Kashef MA, Dehaghani AS, Naeimi S, Fattahi MJ, Ghaderi A. Interleukin-18 gene promoter polymorphisms in women with gestational trophoblastic diseases. J Reprod Med. 2008; 53:853–9.
38. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity. 2005; 95:221–7.
39. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet. 2004;74:765–9.
40. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet. 2003;73:1162–9.
41. Hernesniemi JA, Karhunen PJ, Oksala N, Kähönen M, Levula M, Rontu R, et al. Interleukin 18 gene promoter

polymorphism: a link between hypertension and pre-hospital sudden cardiac death: the Helsinki Sudden Death Study. Eur Heart J. 2009;30:2939–46.

42. Shaw LJ, Bairey Merz CN, Pepine CJ, Reis SE, Bittner V, Kelsey SF, et al. Insights from the NHLBI-Sponsored Women's Ischemia Syndrome Evaluation (WISE) Study: Part I: gender differences in traditional and novel risk factors, symptom evaluation, and gender-optimized diagnostic strategies. J Am Coll Cardiol. 2006;47(3 Suppl):S4–20.
43. Juonala M, Järvisalo MJ, Mäki-Torkko N, Kähönen M, Viikari JS, Raitakari OT. Risk factors identified in childhood and decreased carotid artery elasticity in adulthood: the Cardiovascular Risk in Young Finns Study. Circulation. 2005; 112:1486–93.