Allelic Variants of Upstream Transcription Factor 1 Associate With Carotid Artery Intima-Media Thickness

— The Cardiovascular Risk in Young Finns Study —

Auni Collings, MD[¶]; Salla Höyssä, BM[¶]; Meng Fan, PhD; Mika Kähönen, MD, PhD*; Nina Hutri-Kähönen, MD, PhD^{**}; Jukka Marniemi, PhD[†]; Markus Juonala, MD, PhD^{††}; Jorma S. A. Viikari, MD, PhD[‡]; Olli T. Raitakari, MD, PhD^{‡‡}; Terho J. Lehtimäki, MD, PhD

Background Polymorphisms of the upstream transcription factor 1 (USF1) have been associated with familial combined hyperlipidemia and coronary heart disease. The impact of this gene on subclinical atherosclerosis is unknown. Associations of 3 allelic variants of the *USF1* gene and their haplotypes with carotid artery intima-media thickness (IMT), carotid artery compliance (CAC) and brachial artery flow mediated dilatation (FMD) were studied in a population of Finnish healthy young adults.

Methods and Results The study population comprised 2,281 individuals participating in the Cardiovascular Risk in Young Finns study. IMT, CAC and FMD values were measured by ultrasound examination. Genotypes were analysed using the 5' nuclease assay. A significant difference in IMT was found for *usf1s1* (rs3737787) and *usf1s8* (rs2516838) genotypes (p-values 0.046 and 0.021, respectively). Moreover, there was a significant difference between groups in haplotype 1 and haplotype 2 for IMT (p-values 0.011 and 0.028 respectively). In multivariate stepwise linear regression models adjusted by age, sex, body mass index, systolic and diastolic blood pressures, smoking, C-reactive protein, glucose, high- and low-density lipoprotein-cholesterols and triglycerides there were significant associations for the *usf1s1* minor genotype AA to predict low IMT (p=0.038) and *usf1s8* minor genotype GG to predict high IMT (p=0.003). There was also a significant association for haplotype 2 to predict low IMT in the otherwise similar multivariate model (p=0.006). No associations were found for polymorphisms and CAC, FMD or serum lipids.

Conclusions The rs2516838 and rs3737787 polymorphisms of *USF1* influence the carotid artery IMT, which is a new finding. (Circ J 2008; 72: 1158–1164)

Key Words: Atherosclerosis; Intima-media thickness; Polymorphism; *Usf1*

Atherosclerosis is a progressive multifactorial process that begins in childhood and can develop for decades before clinical manifestations. Commonly known metabolic risk factors include hypercholesterolemia, diabetes and metabolic syndrome. Lipid and glucose metabolism, as well as artery wall inflammation, have been under extensive study in the search for the underlying mechanisms causing risk or progression of the disease.

The upstream transcription factor 1 (USF1) is transcription factor that modulates the expression of more than 40 genes

involved in glucose and lipid metabolism and inflammation, thus being an interesting candidate gene for cardiovascular disease. USF1 binds to specific promoter sequences, E-Boxes with a consensus sequence of CACGTG as a homodimer or as a heterodimer with USF2 transcription factor, thus modulating transcriptional processes (ie, of several genes) involved in inflammatory responses.^{1–4} Familial combined hyperlipidemia (FCHL) is a common genetic dyslipidemia characterized by high levels of cholesterol and/or triglycerides.^{5,6} Recently, specific alleles of the *USF1* gene defined by single nucleotide polymorphisms (SNPs) (especially *usf1s1* and *usf1s2*) were found to associate with FCHL in a Finnish study.² Results were soon replicated in other populations.^{7–9} Functional *USF1* polymorphisms seem to also have an impact on increased risk of coronary vascular disease (CVD) among females.¹⁰

Carotid artery intima-media thickness (IMT) is 1 of the earliest measures of subclinical atherosclerosis and is a predictive factor of future cardiovascular events.^{11,12} IMT is found to correlate with several risk factors of coronary artery disease (CAD),^{13,14} and exposure to these risk factors during childhood has been shown to predict IMT in adulthood.^{15–17} IMT values have been shown to associate with cardiovascular risk factors¹⁸ and predict cardiovascular events such as myocardial infarction and stroke.^{19,20}

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Department of Clinical Chemistry, Centre for Laboratory Medicine, Tampere University Hospital and Tampere University Medical School, *Department of Clinical Physiology, **Department of Paediatrics, Tampere University Hospital, Tampere, †Department of Health and Functional Capacity, Population Research Laboratory, National Public Health Institute, ††Research Centre of Applied and Preventive Cardiovascular Medicine, ‡Department of Medicine and ‡‡Department of Clinical Physiology, University of Turku, Turku, Finland

¶The first two authors contributed equally to the work.

Mailing address: Auni Collings, MD, Department of Clinical Chemistry, Centre for Laboratory Medicine, Tampere University Hospital, PO Box 2000, 33521 Tampere, Finland. E-mail: Auni.Collings@pshp.fi All rights are reserved to the Japanese Circulation Society. For permissions, please e-mail: cj@j-circ.or.jp

Arterial elasticity can be assessed by measuring carotid artery compliance (CAC), which indicates the ability of the arterial wall to expand in response to pulse pressure.²¹ CAC has been associated with several cardiovascular risk factors^{22–24} and it has been suggested to be an independent predictor for cardiovascular events in high-risk individuals.^{25,26} Exposure to conventional cardiovascular risk factors in childhood has been shown to predict decreased CAC in adulthood.²³ Early functional changes of the endothelium can be studied by flow-mediated dilatation (FMD) of the brachial artery.²⁷ Endothelial dysfunction is represented by impaired FMD, which is considered an early result of atherosclerosis.^{27–29}

Although the *USF1* polymorphism *usf1s1* has been connected to FCHL, high triglycerides^{2,9} and low-density lipoprotein-cholesterol (LDL-C)⁹ and polymorphism *usf1s2* to triglycerides and LDL-C in men⁹ and to higher risk of CVD in women,¹⁰ it is still unclear whether this gene is already having an effect on atherosclerosis in the early, subclinical stage. Therefore, in the present study we tested the hypothesis whether variation in *USF1* is associated with some important markers of subclinical atherosclerosis.

The aim was to investigate the association of *USF1* polymorphisms (*usf1s1*=rs3737787, *usf1s2*=rs2073658, and *usf1s8*=rs2516838) and their haplotypes with carotid artery IMT, CAC and brachial artery FMD in 2,281 healthy young Finnish adults who had participated in the ongoing multicenter follow-up study known as The Cardiovascular Risk in Young Finns Study.

Methods

Study Population

The Cardiovascular Risk in Young Finns Study is an ongoing multicenter follow-up study of atherosclerosis risk factors in Finnish children and adolescents. The first cross-sectional survey was in 1980, comprising 3,596 subjects (aged 3–18 years), who were randomly chosen from the national population register.³⁰ At the time of the latest follow-up in year 2001, 2,283 subjects took part (63.5% of the baseline cohort in 1980).^{15,31,32} Subjects gave written consent and local ethics committees approved the study.

General Information and Physical Measurements

Information on cardiovascular risk factors, including smoking habits, was collected by a standardized questionnaire. Data on smoking habits for this study were determined as a 2-level variable: smokers were current smokers and non-smokers had never smoked or were ex-smokers. Systolic and diastolic blood pressures (BPs) were measured with a random-zero sphygmomanometer and the average of 3 measurements was used. Height and weight were measured, and body mass index (BMI, kg/m²) was calculated.

Biochemical Parameters

Blood samples for biochemical analyses (lipids, C-reactive protein (CRP), glucose and homocysteine) were drawn after subjects had fasted overnight. All determinations were done in duplicate in the same laboratory. Plasma triglycerides, as well as total and high-density lipoprotein-cholesterol (HDL-C) concentrations, were analysed by standard enzymatic methods as described previously, and apolipoprotein (apo) A-I and apoB by immunoturbidimetry.^{15,31} LDL-C concentrations were calculated using Friedewald's formula.³³ Sensitive CRP was measured using a latex turbidimetric

immunoassay (Wako Chemicals GmbH, Neuss, Germany) and the lower detection limit was 0.06 mg/L. A microparticle enzyme immunoassay kit (Abbott Laboratories, Tokyo, Japan) was used for homocysteine measurements and glucose was measured enzymatically (glucose dehydrogenase, Olympus Diagnostica GmbH, Hamburg, Germany).

Ultrasound Imaging

Carotid ultrasound studies, measuring IMT, were done with Sequoia 512 ultrasound mainframes (Acuson, CA, USA) with a 13.0 MHz, linear array transducer. The posterior wall of the left common carotid artery was scanned following a standardized protocol, as described in detail elsewhere.^{15,34} In summary, a magnified image from the angle of greatest distance between the lumen–intima and the media–adventitia interface was recorded. A 5-s moving scan, including the beginning of the carotid bifurcation and the common carotid artery, was recorded and stored in digital format on optical discs for subsequent off-line analysis. Scans were analysed by a single reader who was unaware of the subjects' details. The analysis was carried out with ultrasonic callipers. From the clip image, the best quality end-diastolic frame was selected and at least 4 measurements of the common carotid posterior wall were taken, approximately 10 mm proximal to the bifurcation to derive maximal carotid IMT. To assess reproducibility of IMT measurements, 60 subjects were re-examined 3 months after the initial visit. The between-visit coefficient of variation of IMT measurements was 6.4%.

For assessment of CAC, the best quality cardiac cycle from the 5-s clip images was selected. The means of at least 2 measurements of the common carotid artery in end-diastole and end-systole were used as the end-diastolic and the end-systolic diameters. Ultrasound measurements and concomitant brachial BP values were used to calculate $CAC = [(D_s - D_d)/D_d]/(P_s - P_d)$, where D_s is the systolic diameter; D_d the diastolic diameter; P_s the systolic BP and P_d the diastolic BP. The between-visit coefficient of variation was 2.7% for diastolic carotid diameter and 16.3% for CAC.²³

FMD of the brachial artery was measured by ultrasound. The left brachial artery was measured first at rest and then during reactive hyperaemia after a tourniquet had been placed around the forearm and a pressure of 250 mmHg held for 4.5 min. Three measurements of the arterial diameter were made at 3 different time points (40, 60 and 80 s) and the maximum FMD was calculated from the average of these measurements at each time point. The diameter of the vessel during reactive hyperaemia was expressed as relative to the resting scan (100%). The between-visit coefficient of variation for FMD was 26%.²⁹

USF1 Genotyping

DNA was isolated from samples of whole blood, which had been stored frozen at –20°C, with a commercially available kit (Qiagen Inc, Valencia, CA, USA). Samples were genotyped by a 5' nuclease assay in combination with specific fluorogenic TaqMan[®] MGB[™] probes using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences of primers and allele-specific wild-type and variant probes, labelled with the reporter dyes FAM[™] or VIC[®], were deduced from sequences deposited in the GenBank database and synthesized in accordance with Applied Biosystems. A 5-μl reaction volume was used in the PCR reaction containing genomic DNA, 1×Universal Master Mix, 900 nmol/L of

Table 1 Characteristics of the Study Population

	Mean $\pm$ SD
Subjects (M/F)	999/1,239
Age (years)	31.7 $\pm$ 5.0
BMI (kg/m ²) n=2,220	25.1 $\pm$ 4.4
Smoke (N/Y) n=2,178	65%/35%
SBP (mmHg) n=2,205	122.1 $\pm$ 14.5
DBP (mmHg) n=2,205	73.3 $\pm$ 9.0
IMT (mm) n=2,212	0.581 $\pm$ 0.092
Max FMD (%) n=2,058	8.02 $\pm$ 4.430
CAC (%/10 mmHg) n=2,202	2.17 $\pm$ 0.74
TC (mmol/L)	5.17 $\pm$ 0.98
HDL-C (mmol/L)	1.29 $\pm$ 0.32
LDL-C (mmol/L)	3.28 $\pm$ 0.86
TG (mmol/L)	1.34 $\pm$ 0.86
CRP (mg/L)	1.95 $\pm$ 3.99
Gluc (mmol/L)	5.05 $\pm$ 0.84
Hcy (μ mol/L)	9.80 $\pm$ 3.76
Apo A-I (g/L)	1.50 $\pm$ 0.26
Apo B (g/L)	1.06 $\pm$ 0.27

Means and standard deviations ($\pm$ SD) or prevalence%. Subject number n stated when it was less than the whole population of 2,238.

BMI, body mass index; Smoke (N/Y), current smoking (no/yes); SBP, systolic blood pressure; DBP, diastolic blood pressure; IMT, intima-media thickness; Max FMD, maximal brachial artery flow-mediated dilatation; CAC, carotid artery compliance; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TG, triglycerides; CRP, C-reactive protein; Gluc, glucose; Hcy, homocysteine; Apo, apolipoprotein.

each primer and 200 nmol/L of each probe. The reactions were performed in 384-well plates using the standard method. PCR pipetting steps were done with the Tecan® robot with instrument software v4.8 (Tecan Freedom EVO, Männedorf, Switzerland). Allele-specific fluorescence generated from each probe during the PCR amplification was measured with the allelic discrimination analysis module, resulting in the identification of 3 genotypes for both genes. Genotyping did not succeed for 26 (1.1%) subjects. Random duplicates were used as a quality control.

Statistical Analysis

Analysis of variance (ANOVA) was used to test differences in continuous cardiovascular risk factors between the genotype and haplotype groups. Categorical variables were compared using the chi-square test, which was also used to compare the frequencies of the polymorphisms between the study groups. Conformity of the genotype proportion to the Hardy-Weinberg equilibrium was examined in the whole population. Linkage disequilibrium analysis between polymorphisms was carried out using freely distributed software.³⁵ Haplotypes were estimated from the 3 single-nucleotide polymorphisms using the PHASE version 2.1.1 program^{36,37} which uses a Bayesian statistical method for reconstructing haplotypes from population genotype data, and lists the most likely pairs of haplotypes for each individual. Each haplotype was coded as a haplotype number from 1 to 6 according to the allelic content of the haplotype in all, and the results are shown in the order of USF1 single-nucleotide polymorphisms rs3737787, rs2073658 and rs2516838 (eg, haplotypes 1–6 are G-C-C, G-C-G, A-C-C, A-T-C, A-T-G and G-T-G, respectively).

Linear regression analyses without added variables were done to test for possible linear associations between polymorphisms and haplotypes and the vascular parameters (data not shown).

Associations between the polymorphisms and haplotypes

on IMT, CAC and FMD were tested by multivariate linear regression models. The variables were chosen after backward stepwise regression; tested variables included: age, sex, BMI, systolic and diastolic BPs, current smoking, HDL-C, LDL-C, triglycerides, CRP and glucose. Data that did not distribute normally were transformed before analyses: logarithmical transformation for CRP and triglycerides; square root transformation for glucose. Analyses were conducted using the statistical software SPSS (version 14.0, SPSS Inc, Chicago, IL, USA). A p-value of less than 0.05 was considered statistically significant.

Results

The allele frequencies of USF1 were distributed as follows: usf1s1 GG 929 (41.5%), GA 1023 (45.7%), AA 286 (12.5%), usf1s2 CC 930 (41.6%), CT 1023 (45.7%), TT 284 (12.7%) and usf1s8 CC 1173 (52.8%), CG 897 (40.4%) and GG 150 (6.8%). The genotype distributions were in Hardy-Weinberg equilibrium.

Six haplotypes were presumed for the usf1s1 rs3737787, usf1s2 rs2073658 and usf1s8 rs2516838 polymorphisms: G-C-C, G-C-G, A-C-C, A-T-C, A-T-G and G-T-G. In this population there were 5 haplotype pairs each coded categorically as 1=2 copies of the haplotype, 2=1 copy, 3=no copies. Frequencies for the haplotypes were as follows: haplotype 1: 1=321, 2=1077, 3=884, haplotype 2: 1=150, 2=908, 3=1224, haplotype 3: 1=0, 2=0, 3=2281, haplotype 4: 1=287, 2=1060, 3=935 and haplotype 5: 1=0, 2=1, 3=2281.

Characteristics of the study group are shown in Table 1. In the comparison of the list of common risk factor variables between genotype groups for the 3 polymorphisms, no differences were found between any of the conventional risk factors (Table 2). Also, no differences between the groups were found when comparing rare allele carriers of the 3 polymorphisms to common allele homozygotes (data not shown). For the vascular parameters, there was a significant difference between genotypes in IMT for usf1s1 and usf1s8 (p-values 0.046 and 0.021 respectively). No differences were found between groups for CAC or FMD values. Results are shown in Table 3.

As the haplotypes 3 and 5 were mostly all of 1 type, they were not included in further analyses. Testing for differences between risk factors shown in Table 1 in the different haplotype groups revealed significant findings for current smoking and diastolic BP in haplotype 1 (p-values for ANOVA were 0.025 and 0.009 respectively). No differences were found between groups in haplotypes 2 and 4. Table 4 shows the results for the vascular measurements, there was a difference in IMT between groups in haplotypes 1 and 2, p-values 0.011 and 0.028 respectively. CAC or FMD did not differ between groups.

Linear regression analyses were done without added variables to look for linear associations between the vascular parameters and genotypes or haplotypes. Associations were found between usf1s8 and IMT p=0.006 and haplotype 2 and IMT p=0.009. Results for the other genotypes and haplotypes were in the range of p<0.2 except for haplotype 1.

In the backward stepwise linear regression analyses for associations between the polymorphisms or haplotypes and IMT the variables used were: age, sex, BMI, systolic and diastolic BPs, smoking, CRP, glucose, HDL-C, LDL-C and triglycerides. The variables left in the final models including

Table 2 Risk Factor Profile of the Population According to the USF1 Genotypes in Polymorphisms Usf1s1, Usf1s2 and Usf1s8

	Usf1s1 genotypes			p-value	Usf1s2 genotypes			p-value	Usf1s8 genotypes			p-value
	GG	GA	AA		CC	CT	TT		CC	CG	GG	
Subjects (male)	929 (423)	1,023 (448)	286 (128)	0.741	930 (425)	1,023 (449)	284 (128)	0.721	1,173 (520)	897 (410)	150 (64)	0.712
Age (years)	31.7±5.0	31.7±5.0	31.9±5.0	0.741	31.6±5.0	31.7±5.0	31.9±5.0	0.662	31.7±5.0	31.8±4.9	31.6±5.0	0.942
BMI (kg/m ²)	25.1±4.3	25.1±4.5	25.2±4.3	0.833	25.1±4.3	25.1±4.6	25.2±4.2	0.834	25.1±4.5	25.1±4.4	25.3±3.9	0.847
Smoke (N/Y)	567/340	653/338	192/88	0.114	566/342	656/336	191/87	0.080	732/413	569/298	98/50	0.681
SBP (mmHg)	122±15	122±14	123±16	0.498	122±15	122±14	123±16	0.584	122±15	122±14	123±15	0.754
DBP (mmHg)	73±9	73±9	74±10	0.273	73±9	73±10	74±10	0.354	73±9	73±9	74±9	0.370
TC (mmol/L)	5.17±1.01	5.16±0.95	5.20±1.03	0.792	5.17±1.00	5.15±0.95	5.20±1.03	0.798	5.17±0.99	5.17±1.00	5.15±0.90	0.972
HDL-C (mmol/L)	1.28±0.32	1.30±0.32	1.28±0.31	0.321	1.28±0.32	1.30±0.32	1.27±0.31	0.230	1.29±0.32	1.29±0.31	1.31±0.28	0.675
LDL-C (mmol/L)	3.30±0.88	3.26±0.83	3.27±0.90	0.558	3.29±0.88	3.26±0.82	3.27±0.90	0.606	3.28±0.86	3.28±0.86	3.27±0.85	0.994
TG (mmol/L)	1.32±0.75	1.33±0.86	1.46±1.13	0.221	1.32±0.75	1.33±0.86	1.46±1.12	0.170	1.35±0.92	1.35±0.81	1.26±0.70	0.245
CRP (mg/L)	2.03±4.22	1.92±3.99	1.82±3.13	0.785	2.02±4.22	1.89±3.85	1.83±3.14	0.801	1.87±3.75	2.00±4.10	2.17±4.30	0.566
Gluc (mmol/L)	5.06±0.76	5.03±0.89	5.11±0.91	0.386	5.05±0.86	5.12±0.77	5.11±0.91	0.182	5.06±0.83	5.03±0.67	5.05±1.05	0.610
Hcy (μmol/L)	9.85±3.62	9.81±3.95	9.62±3.46	0.511	9.84±3.61	9.83±4.05	9.64±3.47	0.661	9.63±3.37	9.94±4.17	9.95±3.58	0.189
Apo A-I (g/L)	1.49±0.26	1.50±0.25	1.49±0.25	0.349	1.49±0.27	1.50±0.25	1.49±0.24	0.315	1.49±0.26	1.50±0.25	1.50±0.27	0.860
Apo B (g/L)	1.07±0.27	1.06±0.26	1.08±0.30	0.300	1.07±0.27	1.05±0.26	1.08±0.30	0.253	1.07±0.27	1.06±0.26	1.06±0.27	0.935

Analysis of variance for continuous variables, ² test for categorical variables. Log-transformation was performed for skewed variables; CRP, TG and Hcy. BP values given without decimals.

USF, upstream transcription factor; Usf1s1, USF1 polymorphism rs3737787; Usf1s2, USF1 polymorphism rs2073658; Usf1s8, USF1 polymorphism rs2516838; BP, blood pressure. Other abbreviations see in Table 1.

Table 3 Descriptions of the Vascular Variables According to USF1 Genotype

USF1 genotype	Variable		
	Mean IMT (mm)	Max FMD (%)	CAC (%/10 mmHg)
Usf1s1 rs3737787			
GG (n=929)	0.587±0.096	7.98±4.30	2.18±0.74
GA (n=1,023)	0.576±0.090	8.04±4.51	2.18±0.75
AA (n=286)	0.582±0.089	8.08±4.55	2.15±0.75
p-value*	0.046	0.934	0.836
Usf1s2 rs2073658			
CC (n=930)	0.586±0.096	7.98±4.29	2.18±0.74
CT (n=1,023)	0.576±0.090	8.04±4.51	2.17±0.75
TT (n=284)	0.582±0.089	8.06±4.50	2.16±0.75
p-value*	0.060	0.953	0.911
Usf1s8 rs2516838			
CC (n=1,173)	0.577±0.089	8.04±4.42	2.15±0.77
CG (n=897)	0.584±0.093	7.95±4.46	2.19±0.70
GG (n=150)	0.597±0.115	8.35±4.33	2.16±0.77
p-value*	0.021	0.617	0.489

*Analysis of variance.

Abbreviations see in Tables 1,2.

the polymorphisms were: age, sex, BMI and systolic BP. For the haplotype models the final variables were the same as above with the addition of smoking status for haplotypes 1 and 2. Results of these models are shown in Tables 5 and 6.

Age, sex, BMI and systolic BP all had a positive association with IMT for all the polymorphisms. For usf1s1, the addition of the A-allele associated inversely with IMT, so

Table 4 Descriptions of the Vascular Variables According to USF1 Haplotype

USF1 haplotype	Dependent variable		
	Mean IMT (mm)	Max FMD (%)	CAC (%/10 mmHg)
Haplotype 1			
2 copies (n=321)	0.586±0.095	8.14±4.52	2.17±0.72
1 copy (n=1,077)	0.575±0.092	7.83±4.39	2.18±0.76
No copies (n=884)	0.586±0.092	8.14±4.52	2.17±0.72
p-value*	0.011	0.297	0.915
Haplotype 2			
2 copies (n=150)	0.597±0.115	8.35±4.33	2.15±0.77
1 copy (n=908)	0.584±0.093	8.35±4.33	2.19±0.71
No copies (n=1,224)	0.577±0.089	8.00±4.41	2.16±0.76
p-value*	0.028	0.592	0.566
Haplotype 4			
2 copies (n=287)	0.582±0.088	8.06±4.51	2.15±0.75
1 copy (n=1,060)	0.576±0.090	7.99±4.49	2.17±0.75
No copies (n=935)	0.586±0.096	7.98±4.31	2.18±0.74
p-value*	0.057	0.969	0.872

*Analysis of variance.

Haplotype 1, USF1 polymorphisms rs3737787 rs2073658 and rs2516838 (nucleotide sequence G-C-C); haplotype 2, nucleotide sequence (G-C-G) and haplotype 4, nucleotide sequence (A-T-C), respectively. Other abbreviations see in Tables 1,2.

that IMT values fell in the genotype order of GG>GA>AA (p=0.038). The rarer allele associated with lower IMT values. For usf1s8, the effect was to increase IMT values in the genotype order CC>CG>GG (p=0.003); here the rarer allele associated with higher IMT values (Table 5).

Table 5 Multivariate Linear Regression Models (Backward Stepwise, With the Genotype Forced Into the Model) for the Relationships Between Risk Factors and Carotid Artery IMT (mm) in 3 Models Depending on the Different USF1 Polymorphisms Tested

Explanatory variable	Model for <i>usf1s1</i>			Model for <i>usf1s2</i>			Model for <i>usf1s8</i>		
	SE	p-value		SE	p-value		SE	p-value	
<i>Sex (female=1, male=2)</i>	0.011	0.004	0.007	0.011	0.004	0.006	0.013	0.004	0.003
<i>Age (years)</i>	0.005	0.000	<0.001	0.005	0.000	<0.001	0.005	0.000	<0.001
<i>BMI (kg/m²)</i>	0.003	0.000	<0.001	0.003	0.000	<0.001	0.003	0.000	<0.001
<i>SBP (mmHg)</i>	0.001	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.002
<i>Usf1s1 (GG=1, GA=2, AA=3)</i>	-0.006	0.003	0.038	ND	ND	ND	ND	ND	ND
<i>Usf1s2 (CC=1, CT=2, TT=3)</i>	ND	ND	ND	-0.005	0.003	0.069	ND	ND	ND
<i>Usf1s8 (CC=1, CG=2, GG=3)</i>	ND	ND	ND	ND	ND	ND	0.009	0.003	0.003

Variables used in the stepwise analyses were: age, sex, BMI, SBP, DBP, smoking, CRP, Gluc, HDL-C, LDL-C and TG. For the final models adjusted $R^2=13.5\%$, $p<0.001$ for *usf1s1*, $R^2=13.1\%$, $p<0.001$ for *usf1s2* and $R^2=13.5\%$, $p<0.001$ for *usf1s8*.

, unstandardized regression coefficient; SE, standard error; ND, not determined. Other abbreviations see in Tables 1,2.

Table 6 Multivariate Linear Regression Models (Backward Stepwise, With the Haplotype Forced Into the Model) for the Relationships Between Risk Factors and Carotid Artery IMT (mm) in 3 Models Depending on the Different USF1 Haplotypes Tested

Explanatory variable	Model for haplotype 1			Model for haplotype 2			Model for haplotype 4		
	SE	p-value		SE	p-value		SE	p-value	
Sex (female=1, male=2)	0.010	0.004	0.023	0.010	0.004	0.022	0.010	0.004	0.024
Age (years)	0.005	0.000	<0.001	0.005	0.000	<0.001	0.005	0.000	<0.001
BMI (kg/m ²)	0.003	0.000	<0.001	0.003	0.000	<0.001	0.003	0.000	<0.001
SBP (mmHg)	0.000	0.000	0.002	0.000	0.000	0.002	0.000	0.000	0.002
Smoking (no=0, yes=1)	0.008	0.004	0.044	0.008	0.004	0.044	NS	NS	NS
Haplotype 1 (2 copies=1, 1 copy=2, no copies=3)	0.002	0.003	0.482	ND	ND	ND	ND	ND	ND
Haplotype 2 (2 copies=1, 1 copy=2, no copies=3)	ND	ND	ND	-0.008	0.003	0.006	ND	ND	ND
Haplotype 4 (2 copies=1, 1 copy=2, no copies=3)	ND	ND	ND	ND	ND	ND	0.005	0.003	0.054

Variables used in the backward stepwise analyses were: age, sex, BMI, SBP, DBP, smoking, CRP, Gluc, HDL-C, LDL-C and TG. For the final models adjusted $R^2=13.0\%$, $p<0.001$ for haplotype 1, $R^2=13.3\%$, $p<0.001$ for haplotype 2 and $R^2=13.1\%$, $p<0.001$ for haplotype 4. Abbreviations see in Tables 1,2,4,5.

In the final models for the haplotypes, sex, age, BMI and systolic BP were all significantly associated with IMT; smoking also in haplotypes 1 and 2. Results are shown in Table 6. There was a negative association between haplotype 2 and IMT so that values dropped in the order of 2 copies>1 copy>no copies (p -value 0.006). In other words, haplotype 2 predicts higher IMT values.

Discussion

As atherosclerosis is a lifelong disease and exposure to risk factors in childhood predicts high IMT in adulthood, we investigated whether the functional polymorphisms of *USF1* are involved in early atherosclerotic changes in vascular parameters in young Finnish adults.¹⁵ IMT is a good measure of subclinical atherosclerosis and predicts future cardiovascular events.^{12,38} Because variation in *USF1* has been linked to FCHL, metabolic diseases and CHD^{2,10,39} we hypothesised links to early vascular changes.

We found an association between 2 common SNPs of *USF1* and IMT, and an association with IMT was found with 1 haplotype derived from 3 common polymorphisms of *USF1*. This study found that homozygous wild type carriers of *usf1s8* (genotype CC) are likely to have lower mean IMT values and that the presence of the rare allele G raises mean IMT values in a linear manner. The rare allele G in this case is a risk for higher IMT. For *usf1s1* the opposite was true; rare allele carriers were shown to predict lower mean IMT values, but the statistical significance was not as pronounced. In the haplotype analyses we found that haplotype 2 predicts higher IMT values. The haplotype 2 is a nucleotide sequence G-C-G for polymorphisms *usf1s1*, *usf1s2* and *usf1s8*, respectively. It may have been expected that *USF1*,

via the influence it has on blood biochemical analytes, would associate with vascular parameters. In our stepwise models, however, CRP, glucose, HDL-C, LDL-C and triglycerides were not significant predictors for IMT. And in our final model adjusted for sex, age, BMI and systolic BP, there was a direct association between genotype or haplotype and IMT. This may suggest that the gene has direct influence on the vascular wall independent of the measured serum biochemistry it affects.

A sex-dependent association has been found between the common allele of *usf1s1* and triglycerides and related metabolic traits in Dutch males of FCHL families and in US white males.⁴⁰ Also, in a different population of healthy white American adults (the CARDIA study), the rare allele of *usf1s1* was associated with lower total cholesterol and lower LDL-C, but also with decreased risk of coronary artery calcium. As there was little attenuation of this association when adjusted for cholesterol, it was suggested that *USF1* might influence CVD risk via atherogenic pathways other than lipids.⁴¹ There was no sex-dependent trait in our population, but the rare allele of *usf1s1* associating to lower IMT was in the same direction as in the CARDIA study for decreased risk for calcification. In previous studies linking the gene to cardiovascular disease, the viewpoint has been more on the gene associating with traits predisposing to CVD^{2,8,39} and the association has been via its effect on lipid profiles or other metabolic conditions that associate with CVD. In a recent study the direct effect of *USF1* on CVD was assessed and a sex-specific association with CVD and mortality was found for 2 polymorphisms.¹⁰ In the same study the question was raised whether the gene variants have other ways of affecting CVD risk than via lipid parameters. We found that the variants indeed seem to directly

influence IMT and the lipid values were not significant variables in our models. The underlying mechanism for this phenomenon has yet to be determined. It has been reported that in relatives of FCHL patients there is an increased risk for CVD mortality, indicating that the predetermined genetic factors in these individuals may effect the disease progression independently of risk factors.⁴² However, *USF1* is biologically highly relevant, because it encodes a ubiquitously expressed transcriptional regulator of several genes, such as apoE,⁴³ CRP,⁴⁴ plasminogen activator inhibitor-1⁴⁵ and osteopontin,⁴⁶ which are functionally important for lipid accumulation,⁴⁷ inflammation, and thrombosis. All these proteins have been recovered directly from atherosclerotic plaques of artery wall^{48–51} and all of them have also been connected to the development of early atherosclerosis.^{52–55} Also, it has been speculated that gene–gene or gene–environment interactions may explain the heterogeneity of the results in studies of *USF1* polymorphisms as risk factors for atherosclerosis;⁴¹ and that alterations in the expression or function of *USF1* may influence other metabolic routes in addition to lipids⁴⁰ and act in an atherogenic manner.

The expression of *USF1* has been studied in fat tissue samples of familial combined hypercholesterolemia patients.² The *USF1* haplotype that was studied both up- and downregulated genes and seemed to affect expression profiles in the fat biopsy samples.² It was suggested that potential effects of the *USF1* risk alleles on the regulation of gene transcription may result from tissue- or cell-type specific differences, which may also be affected by local stimuli.² In the present study, we only tested for 3 polymorphisms and other significant associations may be found when more data accumulates for the other known polymorphisms of *USF1* and on the effects they may have on the function of the transcription factor. We cannot on the basis of these data rule out any possible associations other known polymorphism may have.

As a limitation, our study population was young, with no manifestations of atherosclerosis. Age is a significant predicting factor of the vascular parameters used and the results may be different for an older population with manifest atherosclerosis. This study, however, aimed to look for associations in the subclinical stage of atherosclerosis.

The present study is the first to study the influence of polymorphisms of transcription factor *USF1* on vascular parameters used in assessing atherosclerosis (ie, carotid artery IMT and CAC and brachial artery FMD). Connections were found between 2 polymorphisms and 1 haplotype on IMT. The rare allele A of *usf1s1* associates with low IMT and the rare allele G of *usf1s8* with higher IMT values. One haplotype also associated with lower IMT values and the haplotype included the rare allele of *usf1s1* and the wild type allele of *usf1s8*. The association seems to be independent of the lipid or glucose status, because they were eliminated as variables in the multivariate model, which would indicate that the polymorphisms directly influence IMT. There were no associations found for polymorphisms and CAC or FMD. We have previously found a relatively large within-subject long-term variation in CAC²³ and FMD measurements,²⁹ which are, however, in agreement with prior reports.^{56–58} This variability offers one explanation why *USF1* gene variation was associated only with IMT in the present study.

In conclusion, the polymorphisms *usf1s1* rs3737787 and *usf1s8* rs2516838 and one of the studied haplotypes affected carotid artery IMT in young adults.

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