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A common interleukin 18 haplotype is associated with higher body mass index in subjects with diabetes and coronary heart disease

Simon R. Thompson[a], Julie Sanders[b], Jeffrey W. Stephens[c], George J. Miller[d], Steve E. Humphries[a,*]

[a]*Department of Cardiovascular Genetics, The Rayne Institute, WC1E 6JF London, UK*
[b]*Division of Surgical and Interventional Science, The Heart Hospital, UCLH NHS Foundation Trust, W1G 8PH London, UK*
[c]*The Medical School, University of Wales Swansea, SA2 8PP Swansea, UK*
[d]*Department of Environmental and Preventive Medicine, Medical Research Council Cardiovascular Group, Wolfson Institute of Preventive Medicine, EC1M 6BQ London, UK*

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Abstract

The pleiotropic proinflammatory cytokine, interleukin 18, plays a role in innate immunity and, based on mouse models, influences obesity. We investigated variation within the *IL18* gene and its effect on markers of the metabolic syndrome. A tagging single nucleotide polymorphism set of 5 SNPs for the gene encoding interleukin 18 was selected and genotype was determined in 3 separate studies. In 2775 healthy middle-aged men, 6 common haplotypes were seen, but none was associated with body mass index (BMI). In 439 patients who underwent coronary artery bypass graft surgery, Hap2 (frequency, 22%) was present at a lower frequency than in healthy subjects and was associated with higher mean BMI compared with Hap1 ($P = .011$). In 483 men with type 2 diabetes mellitus, Hap2 was again associated with a higher haplotypic mean BMI ($P = .002$). Those homozygous for Hap2 had a BMI of 31.2 (1.3) kg/m^2, mean (SE), compared with 28.3 (1.0) kg/m^2 in those not carrying a copy of Hap2. No single SNP could fully explain the effects seen. Therefore, variation within *IL18*, previously shown to be associated with lower *IL18* levels, is influencing measures of obesity both in men with type 2 diabetes mellitus and those with advanced coronary heart disease.

1. Introduction

Interleukin 18 (IL-18) is a pleiotropic cytokine involved in both innate and adaptive immune responses [1,2]. Originally identified as an interferon γ–inducing factor [3], it stimulates interferon γ production in T lymphocytes and natural killer cells, both of which are essential in atherosclerotic plaque progression and stability [4,5]. Increased IL-18 expression is seen in atherosclerotic plaques and is associated with plaque instability [6,7]. The benefit of IL-18 inhibition in animal models has further demonstrated its influence on plaque progression and composition [8,9]. Large studies have shown that plasma IL-18 levels are an independent predictor of coronary events in healthy, middle-aged European men [10], and recently, variation within the *IL18* gene has been shown to influence circulating levels of IL-18 and clinical outcome in subjects with coronary artery disease [11], as well as to affect IL-18 production capability by monocytes [12].

Obesity is accompanied by low-grade inflammation and there are data to suggest that some circulating inflammatory mediators, including IL-18 [13], are released from fat tissue [14,15]. Serum IL-18 levels correlate with body mass index (BMI) and waist circumference, independently of obesity and hyperinsulinemia [16]. Interleukin 18 has also been implicated as an adipogenic cytokine, with *il18*$^{-/-}$ mice having twice the percentage body fat of their wild-type littermates, as well as developing several features characteristic of the metabolic syndrome [17]. This, among other data, suggests a new role for IL-18 in energy homeostasis. We therefore investigated the effect of variation in *IL18* on BMI and other measures of obesity in healthy middle-aged men participating in the second Northwick Park Heart Study (NPHSII), in a group of patients with diabetes mellitus

* Corresponding author. Tel.: +44 0207 679 6962; fax: +44 0207 679 6212.
E-mail address: rmhaseh@ucl.ac.uk (S.E. Humphries).

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(University College London Diabetes and Cardiovascular Study [UDACS]), and in a group with coronary heart disease (CHD) undergoing coronary artery bypass graft (CABG) surgery.

2. Methods

2.1. Study subjects

2.1.1. The second Northwick Park Heart Study

The NPHSII is a large prospective (15 years of follow-up) study of 3012 healthy white European middle-aged men recruited from 9 general medical practices in the United Kingdom designed to examine risk factors for CHD. All eligible subjects were free of a history of unstable angina, myocardial infarction or evidence of silent infarction, coronary surgery, aspirin or anticoagulant therapy, cardiovascular disease, malignancy (except skin cancer other than melanoma), or any condition precluding informed consent [18,19]. In the data presented herein, those who presented with CHD during follow-up were excluded. Ethical approval was granted by the institutional ethical committee and written informed consent was obtained before recruitment.

2.1.2. Coronary artery bypass graft study

This study is described elsewhere [20,21]. Briefly, 439 participants were recruited prospectively from those undergoing first-time elective CABG between October 1999 and July 2003. Participants with evidence of an active inflammatory or immunomodulatory disease or who were receiving anti-inflammatory agents other than aspirin were excluded before recruitment. All participants were taking aspirin (75 mg/d) and ceased 7 days before surgery. All other drugs were withheld on the morning before surgery. Ethical approval was granted by the institutional ethical committee and written informed consent was obtained before recruitment.

2.1.3. University College London Diabetes and Cardiovascular Study

The UDACS participants were 1011 consecutive patients recruited from the diabetes clinic at University College London Hospitals National Health Service Trust between the years 2001 and 2002 [22,23]. All participants had diabetes according to World Health Organization criteria [24]. Ethical approval was granted by the institutional ethical committee and written informed consent was obtained before recruitment.

2.2. Tagging single nucleotide polymorphism identification

Interleukin 18 resequencing data from Innate Immunity PGA (IIPGA; http://innateimmunity.net) was used in conjunction with a haplotype-tagging single nucleotide polymorphism (tSNP) program, tagSNPs.exe, a variant of the Excoffier-Slatkin E-M algorithm, to pick haplotype-tagging polymorphisms [25].

2.3. Interleukin 18 tSNP genotyping

Both restriction fragment length polymorphism (RFLP) and TaqMAN techniques were used. RFLP sequence amplification was performed by using polymerase chain reaction (PCR) (for primer pairs see Supplementary Table 1), PCR product was then digested with the relevant restriction enzyme and product size was determined by microtiter array diagonal gel electrophoresis [26]. Results were entered by 2 independent observers blinded to clinical details. Polymerase chain reaction and TaqMAN probes were designed by Applied Biosystems (Foster City, CA) (see Supplementary Table 2). Reactions were performed in 384-well microplates with Thermo MBS384 thermal cyclers. Fluorescence was measured with an ABI Prism 7000 sequence detection system and analyzed with the ABI Prism 7000 SDS software version 2.1.

2.4. Statistical analysis

All statistical analysis was performed by using SPSS version 12.0.1 (SPSS, Chicago, IL). A χ^2 test compared observed numbers of each genotype with those expected for a population in Hardy-Weinberg equilibrium. When using nonnormally distributed data the nonparametric Mann-Whitney test was used, or data were transformed to attain normalized data and an independent t test, analysis of variance, or χ^2 test was used where appropriate. P values less than .05 were considered significant. Linkage disequilibrium (LD) between sites was estimated by using Haploview version 3.0 (www.broad.mit.edu/mpg/haploview); data are presented as D'. Individual haplotype pairs were inferred by using PL-EM version 1.0 [27]. This program uses a partition-ligation (PL) strategy together with the expectation-maximization (EM) algorithm to reconstruct individual haplotypes based on unphased genotype data. Thesias version 2 [28] was used mainly for haplotype analysis with select associations further analyzed with SimHap Beta Release v2.1B [29]. P values presented are those given by Thesias. All haplotype associations presented were seen to act additively. Adjustment of variables was not carried out unless factors were seen to differ significantly between groups and could plausibly influence the association being tested. Both study samples contain participants of differing ethnic groups. However, there was no suggestion that the associations presented were ethnicity dependent (data not shown); therefore, all data from ethnic groups were combined to maintain group numbers. Ethnic groups were analyzed separately only when polymorphism or haplotype frequencies were being compared, an instance where ethnic origin would likely cause bias in the result.

3. Results

3.1. Baseline characteristics

The baseline characteristics of the 3 separate study groups differed greatly (see Table 1). NPHSII is a healthy

Table 1
Baseline characteristics of the 3 study groups

	NPHSII (n = 2519)	CABG (n = 439)	P^1	UDACS (n = 1011)	P^2
Age	56.1 (3.5)	64.8 (8.9)	<.001	61.8 (13.3)	<.001
BMI (kg/m^2)	26.4 (3.5)	28.5 (4.6)	<.001	28.7 (5.7)	<.001
SBP (mm Hg)	138 (19.2)	–	–	140 (20.5)	.072
DBP (mm Hg)	84.5 (11.4)	–	–	80.3 (11.2)	<.001
Cholesterol (mmol/L)	5.7 (1.0)	4.8 (1.1)	<.001	5.1 (1.1)	<.001
TG (mmol/L)	2.1 (1.3)	–	–	2.1 (1.6)	.014
LDL (mmol/L)	4.0 (1.0)	2.6 (0.9)	–	2.8 (1.0)	–
HDL (mmol/L)	0.85 (0.26)	1.3 (0.4)	–	1.4 (0.5)	–
CRP (mg/L)	5.5 (7.9)	4.0 (4.3)	–	2.2 (2.0)	–
Caucasian (%)	98.8	88.0	<.001	79.0	<.001
Diabetic (%)	–	18.1	<.001	–	–
Ever smoked (%)	58.8	74.9	<.001	47.1	<.001
Male (%)	100	81.8	<.001	62.4	<.001
Type I diabetic (%)	–		–	18.5	–
Type II diabetic (%)	–		–	81.5	–

Values are expressed as mean (SD) unless otherwise indicated. P^1, CABG vs NPHSII, P^2, UDACS vs NPHSII. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CRP, C-reactive protein.

cohort; therefore, as expected, the participants had lower BMI and systolic blood pressure than patients with diabetes mellitus (UDACS) or CHD (CABG). However, on average, those in NPHSII had higher total cholesterol than those in CABG and UDACS, most likely because of lipid-lowering treatment use in both patient groups.

3.2. Tagging SNP selection and validation

A tSNP set comprising IIPGA SNPs −9731 G>T, −5848 T>C, +4860 A>C, +8855 T>A, and +11015 A>C (rs1946519, rs2043055, rs549908, rs360729, rs3882891, respectively) was selected, based on haplotypes derived from IIPGA Caucasian resequencing data. The set was estimated to capture greater than 95% of variation within the 21-kilobase *IL18* region, stretching from 1 kilobase pair upstream to 300 base pairs downstream of the gene. The set comprises 3 intronic SNPs, a proximal promoter variant, and one synonymous SNP within exon 4. Genotypes for all 5 SNPs were determined in all study groups. Only one SNP deviated from the genotype counts expected by Hardy-Weinberg equilibrium, +8855 T>A, with more heterozygotes seen than expected in NPHSII only (P = .044). Because of the number of SNPs and studies analyzed, this could be due to chance alone. Linkage disequilibrium was significant and high across the whole region (see Fig. 1), ranging from D' = 0.70 to D' = 0.99 in NPHSII.

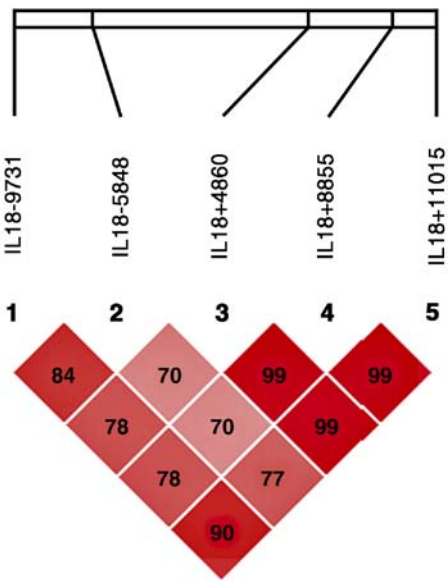

Fig. 1. Linkage disequilibrium (D') values between the 5 SNPs in NPHSII.

Haplotypes inferred from all study groups were largely similar in frequency and identity to those reported by IIPGA, suggesting that the genomic variation and structure of *IL18* was not significantly different within each study group from the populations genotyped by IIPGA. Therefore, the tSNP set derived from IIPGA data was effective and translated well to the study groups, implying that with the selected 5 polymorphisms, more than 95% of genetic variation within *IL18* was captured.

3.3. Frequency variations

When analysis was confined to white Europeans only, a number of the polymorphisms genotyped differed in frequency between the study groups (see Table 2). Both −9731T and +11015C were seen at a significantly higher frequency in CABG and UDACS compared with NPHSII (all P < .04). The only other allele seen to differ significantly in frequency was +8855 T>A in CABG; however, the allele frequency was greatly different from that observed in UDACS (where it closely matched NPHSII), suggesting that this difference in allele frequency was not associated with disease and may be the result of type I error.

Haplotypes were inferred separately in all study groups by using PL-EM. In total, 6 different common (greater than 2% in frequency) haplotypes were observed (see Table 3).

Table 2
Rare allele frequencies (±95% CI) in NPHSII, CABG (Caucasians only), and UDACS (Caucasians with T2DM only)

Polymorphism	NPHSII	CABG		UDACS	
	Rare allele frequency	Rare allele frequency	P^1	Rare allele frequency	P^2
−9731 G>T	0.388 (0.36-0.39)	0.419 (0.38-0.46)	.035	0.422 (0.39-0.45)	.005
−5848 T>C	0.375 (0.36-0.39)	0.365 (0.33-0.40)	.620	0.380 (0.35-0.41)	.053
+4860 A>C	0.295 (0.28-0.31)	0.326 (0.29-0.36)	.095	0.303 (0.28-0.33)	.555
+8855 T>A	0.296 (0.28-0.31)	0.341 (0.31-0.38)	.016	0.302 (0.28-0.33)	.669
+11015 A>C	0.395 (0.38-0.41)	0.439 (0.40-0.48)	.027	0.436 (0.41-0.46)	.011

P^1, CABG vs NPHSII; P^2, UDACS vs NPHSII.

A significant difference (both $P < .04$) in frequency of Hap2 was seen between NPHSII and both CABG and UDACS, with it being at a lower frequency in the 2 diseased cohorts. Conversely, Hap4 was seen at a significantly higher frequency in UDACS than NPHSII only ($P = .008$), mirroring in part the difference in allele frequency described above.

3.4. Interleukin 18 genotype is associated with differences in BMI and waist circumference

Having observed significant differences in both allele and haplotype frequencies among the study groups, we investigated the effect of *IL18* variation on intermediate phenotypes common to CHD and type 2 diabetes mellitus (T2DM). The effect of *IL18* variation on BMI was investigated, as it was significantly higher in the 2 patient groups compared with NPHSII (see Table 1).

3.4.1. Second Northwick Park Heart Study

Single SNP analysis showed no significant associations with BMI (see Table 4) in the total sample or in those who developed diabetes during follow-up, although this number is small (n = 132) (all $P > .28$). No significant associations were seen with haplotype analysis in the total sample (see Table 4), or in those who developed diabetes during follow-up.

3.4.2. Coronary artery bypass graft

In the subjects undergoing CABG, single SNP analysis showed BMI differed by −5848 T>C genotype but not for any other SNP (see Table 4). Mean BMI by −5848 T>C genotype showed a non–"dose-dependent" pattern, indicative of a more complex haplotypic mechanism. Overall, *IL18* haplotypes were not significantly associated with differences in BMI ($P = .26$); however, Hap2 was associated with higher BMI ($P = .011$) in the total sample. Both the single SNP and the haplotype effect were seen in men ($P = .045$ and $P = .009$, respectively) but not in women ($P = .90$ and $P = .55$, respectively). However, the number of women within CABG was low and this sample is inadequately powered to pick up the effect size seen in men.

3.4.3. University College London Diabetes and Cardiovascular Study

None of the single SNPs alone were significantly associated with BMI (all $P > .25$) in the total sample. Mean BMI (±SE) was significantly higher ($P < .001$) in those with T2DM (29.2 [0.20] kg/m^2, n = 783) compared with those without (26.5 [0.38] kg/m^2, n = 179). Inflammatory mechanisms appear to play a prominent role in the development of this condition, and analysis was therefore focused on these subjects, predicting that the effect of IL-18 would be greater in them than in others. Of the 5 SNPs, only −5848 T>C was significantly associated with differences in BMI in men with T2DM ($P = .024$). Those homozygous for the rare allele had, on average, a 10% lower BMI, consistent with the findings in CABG. This SNP effect was not statistically significant in women with T2DM ($P = .86$); however, those homozygous for the rare allele had a lower mean BMI than those homozygous for the common allele.

IL18 haplotypes were not significantly associated with BMI in the total sample ($P = .26$); however, Hap2 was again associated with a significantly higher BMI than Hap1 ($P = .041$). This association was seen in men ($P = .015$) but not in women ($P = .72$). In men with T2DM, Hap2 was also significantly associated with a 6% higher haplotypic BMI than those with Hap1 ($P = .0007$) (see Fig. 2). Analysis using SimHap demonstrated that this effect remained when analyzed by counts of Hap2 (see Fig. 3).

Table 3
Haplotype frequencies (±95% CI) in NPHSII, CABG (Caucasians only), and UDACS (Caucasians with T2DM only)

Haplotype		NPHSII	CABG		UDACS	
		Haplotype frequency	Haplotype frequency	P^1	Haplotype frequency	P^2
12111	Hap1	0.324 (0.31-0.34)	0.278 (0.24-0.32)	–	0.324 (0.29-0.35)	–
11111	Hap2	0.260 (0.25-0.27)	0.223 (0.19-0.25)	.037	0.216 (0.19-0.24)	.035
21222	Hap3	0.257 (0.24-0.27)	0.250 (0.22-0.29)	.330	0.270 (0.24-0.30)	.595
21112	Hap4	0.097 (0.09-0.11)	0.091 (0.07-0.11)	.346	0.129 (0.11-0.15)	.008
12222	Hap5	0.030 (0.03-0.04)	0.036 (0.02-0.05)	.684	0.029 (0.02-0.04)	.938
22111	Hap6	0.022 (0.02-0.03)	0.029 (0.02-0.04)	.312	0.023 (0.01-0.03)	.904

Inferred using PL-EM. Haplotypes read 5′ to 3′: 1 indicates common allele; 2, rare allele. P^1, CABG vs NPHSII; P^2, UDACS vs NPHSII.

Table 4
Genotypic mean (SD) [n] and haplotypic mean (SE) BMI (kg/m^2) for NPHSII, CABG, and UDACS (T2DM only)

		NPHSII	*P*	CABG	*P*	UDACS	*P*
−9731	GG	26.4 (3.5) [1089]	.58	28.6 (4.3) [162]	.74	29.2 (5.2) [259]	.46
	GT	26.5 (3.4) [1253]		28.3 (4.7) [235]		29.4 (5.9) [386]	
	TT	26.6 (3.8) [412]		28.8 (5) [71]		28.8 (5.8) [123]	
−5848	TT	26.6 (3.4) [1090]	.16	29.2 (4.6) [173]	.01	29.7 (5.7) [267]	.07
	TC	26.3 (3.5) [1272]		27.7 (4.5) [230]		29.3 (6) [356]	
	CC	26.5 (3.5) [386]		29.2 (4.8) [67]		28.3 (4.4) [142]	
+105	AA	26.5 (3.6) [1394]	.81	28.7 (4.3) [217]	.65	29 (5.3) [392]	.35
	AC	26.5 (3.4) [1112]		28.3 (5.2) [208]		29.6 (6.1) [311]	
	CC	26.3 (3.3) [256]		28.4 (2.8) [44]		28.7 (5.2) [64]	
+8855	TT	26.5 (3.6) [1348]	.92	28.6 (4.3) [207]	.77	29 (5.3) [396]	.30
	TA	26.5 (3.4) [1058]		28.4 (5.2) [217]		29.6 (6.2) [309]	
	AA	26.4 (3.3) [255]		28.4 (2.8) [44]		28.6 (5.1) [65]	
+11015	AA	26.4 (3.5) [1036]	.77	28.7 (4) [144]	.52	29.2 (5.5) [248]	.84
	AC	26.5 (3.5) [1298]		28.6 (5) [247]		29.4 (5.8) [383]	
	CC	26.4 (3.4) [453]		27.8 (4.5) [75]		29.2 (5.9) [140]	
Hap1	0	26.3 (1.0)	.35	28.6 (1.0)	.07	29.1 (1.0)	.07
	1	25.3 (1.0)		27.5 (1.0)		28.1 (1.0)	
	2	25.3 (1.0)		29.6 (1.0)		28 (1.0)	
Hap2	0	26.3 (1.0)	.2	27.7 (1.0)	.07	28.4 (1.0)	.002
	1	25.3 (1.0)		28.7 (1.0)		29.4 (1.0)	
	2	27.3 (1.0)		28.8 (1.1)		29.5 (1.0)	
Hap3	0	26.2 (1.0)	.91	28.4 (1.0)	.52	28.7 (1.0)	.39
	1	27.2 (1.0)		27.3 (1.0)		29.7 (1.0)	
	2	25.2 (1.0)		29.4 (1.0)		27.6 (1.0)	
Hap4	0	26.2 (1.0)	.18	28.2 (1.0)	.50	28.8 (1.0)	.66
	1	27.2 (1.0)		27.2 (1.0)		27.8 (1.0)	
	2	25.2 (1.0)		27.1 (1.1)		27.7 (1.0)	
Hap5	0	26.2 (1.0)	.63	28.2 (1.0)	.50	28.7 (1.0)	.76
	1	25.2 (1.0)		27.1 (1.1)		29.7 (1.0)	
	2	25.2 (1.1)		–		29.7 (1.2)	
Hap6	0	26.2 (1.0)	.18	28.1 (1.0)	.20	28.8 (1.0)	.56
	1	27.2 (1.0)		29.1 (1.1)		27.7 (1.0)	
	2	25.2 (1.1)		–		–	

P generated by analysis of variance.

No significant association was observed in the remainder of the male participants ($P = .104$) or in female participants with T2DM ($P = .225$).

Body mass index and waist circumference are highly correlated measures of obesity [16]; we therefore investigated whether *IL18* variation was associated with waist circumference in the small group within UDACS who had measures (n = 192). None of the tSNPs alone were associated with waist circumference (all $P > .25$). *IL18* haplotypes did not affect waist circumference overall ($P = .20$). However, in those with T2DM, mean haplotypic waist circumference was significantly higher in those with Hap2 than with Hap1 ($P = .004$), with those homozygous for Hap2 having a mean (SE) waist circumference of 112.8 (5.8) cm compared with 99.7 (1.4) cm in those with no copies of Hap2.

4. Discussion

We report for the first time the influence of genetic variation within *IL18* on BMI in 2 independent studies. The size of these effects is large and is likely to be of clinical significance. In both patients with CHD and those with T2DM, Hap2 was associated with significantly higher BMI (14% and 17%, respectively) compared with Hap1. From published data determining the relationship between BMI and CHD mortality [30], it can be estimated that subjects homozygous for Hap2, who represent around 5% of the population would be at a 1.2-fold higher risk of CHD compared with Hap1 homozygous men.

Recently, Tiret et al [11] highlighted the role of the *IL18* gene in cardiovascular disease, demonstrating that *IL18* haplotypes caused variation in IL-18 serum levels and were associated with cardiovascular mortality. The 5 *IL18* tSNPs genotyped here were not the same as those used by Tiret et al. However, because both are studies in Caucasian subjects, it can be deduced, using IIPGA *IL18* resequencing (http://innateimmunity.net/), that Hap2 represents that haplotype previously found to be associated with lower IL-18 levels and a protective effect on risk [11]. Therefore, the same *IL18* haplotype has been shown to be associated with CHD risk itself and various CHD risk factors (IL-18, BMI, and

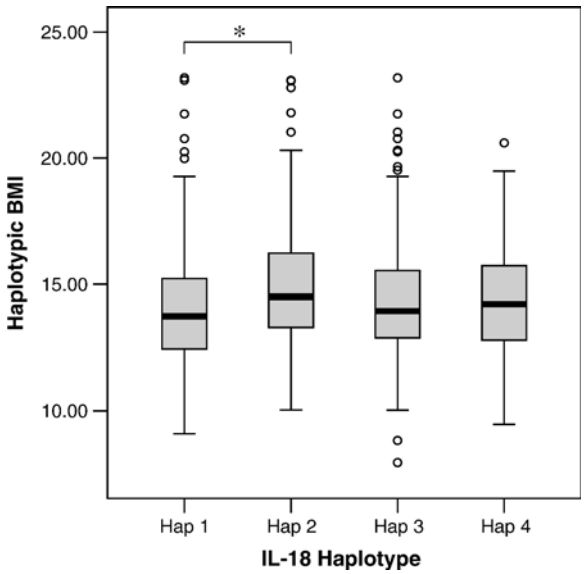

Fig. 2. Haplotypic BMI (kg/m^2) by *IL18* haplotype in UDACS male patients with T2DM. *P = .0007.

waist circumference) in 3 separate studies, strongly suggesting that this haplotype is functionally distinct.

The mechanism whereby genetic variation that influences the levels of IL-18 is involved in the development of CHD, diabetes, and adiposity is likely to be affected through inflammation and activated innate immunity [31-33]. Animal models support the role of IL-18 in plaque progression and stability [8,9] and show the benefits of overexpression of its IL-18 binding protein (its natural inhibitor) [34]. In the AtheroGene study, IL-18 was one of the strongest predictors of future cardiovascular events in those with stable and unstable angina [10]. The 2 "diseased" study groups used here, one representative of advanced cardiovascular disease (CABG at baseline) and the other of diabetes, show many similarities in the effect of variation within *IL18*.

Initially, it appears paradoxical that a haplotype associated with lower IL-18 levels and a protective effect on CHD risk is also associated with higher BMI. However, data recently presented on the *il18*$^{-/-}$ mouse suggested that IL-18 was a satiety factor and was more likely having its effect on the hypothalamus rather than on adipose tissue itself [17]. Therefore, it seems possible that IL-18's effect on BMI (and its possible causal role in atherogenesis) and T2DM/metabolic syndrome represent 2 distinct pathways. By realizing this, it is possible to rationalize Hap2 being associated with increased BMI, yet being a protective effect on risk, as 2 separate effects. However, the details of energy homeostasis between species are likely to differ and we must be cautious when interpreting findings in one species and applying it to another.

The associations presented here are made in groups of participants with either advanced atherosclerosis or diabetes, so perturbation of the processes by which IL-18 may influence BMI due to disease and inflammatory mechanisms is possible. If this were the case, then the effect on BMI being strongest in UDACS participants with T2DM appears consistent, because IL-6 levels and, consequently, inflammation are significantly higher in those with T2DM compared with those with type 1 diabetes mellitus [35] (in UDACS, 4.9 and 2.7 pg/mL, respectively; $P < .001$). This may also explain why the association is not seen in NPHSII, as this is a healthy cohort.

The effect of *IL18* on BMI was seen more consistently in men than in women. That the effect was seen in women to some degree suggests that Hap2 is influencing BMI in women, however, because of the low numbers of women in both CABG and UDACS, both studies lacked the statistical power to consistently see the effect. It is also possible that the nature of *IL18* involvement in adipose metabolism could differ between the sexes. There are sex differences in the control of energy homeostasis and body fat distribution [36], and because visceral and subcutaneous fat are metabolically distinct [37] and the proportions of each differ between the sexes [36], the factors influencing adiposity will also differ between the sexes. Furthermore, both groups studied were diseased cohorts and the sex differences could be exacerbated by disease.

The variant that distinguishes between Hap2 and Hap1 is IIPGA −5848 T>C. The single SNP association data presented here mirror the haplotype association data to some degree, further illustrating the resilience of the haplotype association. However, it also demonstrates that single SNPs will not always confirm or replicate associations seen with haplotypes, and vice versa. Those homozygous for a single allele will not always be homozygous for a single haplotype; therefore, often single SNP analysis is too simplistic. This problem is greater when dealing with tSNPs, which, by their very definition, are chosen because of their high LD with other variants within and outside the

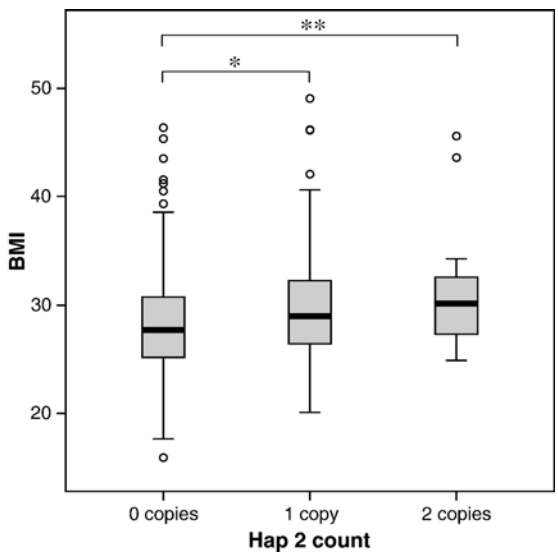

Fig. 3. BMI (kg/m^2) by counts of Hap2 in men with T2DM. Overall P = .005; *P = .0095; **P = .025.

region of interest. The use of other *IL18* SNPs in an attempt to "break down" the associated haplotypes, or an investigation of the associations in other ethnic groups (in which the haplotype structure and pattern of *IL18* LD are radically different), could provide further information on identifying the likely functional SNPs. However, only functional studies that directly test the effect of that particular base change will discriminate between it and other variants that are in high LD with it.

A limitation of this study is the small group sizes of CABG and UDACS; inevitably, when using a haplotypic approach, the amount of data used in analyses is less than that used in a candidate SNP approach. However, using tSNPs in conjunction with haplotype analysis captures near-total genetic variation within the region studied, and therefore the power to pick up genetic effects is increased. The fact that the major results presented have been replicated in 2 separate studies and tie-in with previously published genetic associations strongly suggest that these observations are not due to chance.

In conclusion, the effect of *IL18* on BMI was large and of a degree that could impact on individual CVD risk, implying IL-18 may play a role in obesity, the metabolic syndrome, and CVD, and could be one of the unifying links between all three.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2006.12.015.

References

[1] Dinarello CA. Interleukin-18. Methods 1999;19:121-32.
[2] Dinarello CA, Fantuzzi G. Interleukin-18 and host defense against infection. J Infect Dis 2003;187(Suppl 2):S370-84.
[3] Okamura H, Tsutsi H, Komatsu T, et al. Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature 1995;378:88-91.
[4] Gupta S, Pablo AM, Jiang X, et al. IFN-gamma potentiates atherosclerosis in ApoE knock-out mice. J Clin Invest 1997;99:2752-61.
[5] Libby P. Molecular bases of the acute coronary syndromes. Circulation 1995;91:2844-50.
[6] Mallat Z, Corbaz A, Scoazec A, et al. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. Circulation 2001;104:1598-603.
[7] Gerdes N, Sukhova GK, Libby P, et al. Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for atherogenesis. J Exp Med 2002;195:245-57.
[8] Whitman SC, Ravisankar P, Daugherty A. Interleukin-18 enhances atherosclerosis in apolipoprotein E(−/−) mice through release of interferon-gamma. Circ Res 2002;90:E34-8.
[9] de Nooijer R, der Thusen JH, Verkleij CJ, et al. Overexpression of IL-18 decreases intimal collagen content and promotes a vulnerable plaque phenotype in apolipoprotein-E–deficient mice. Arterioscler Thromb Vasc Biol 2004;24:2313-9.
[10] Blankenberg S, Luc G, Ducimetiere P, et al. Interleukin-18 and the risk of coronary heart disease in European men: the Prospective Epidemiological Study of Myocardial Infarction (PRIME). Circulation 2003;108:2453-9.
[11] Tiret L, Godefroy T, Lubos E, 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.
[12] Arimitsu J, Hirano T, Higa S, et al. IL-18 gene polymorphisms affect IL-18 production capability by monocytes. Biochem Biophys Res Commun 2006;342:1413-6.
[13] Skurk T, Kolb H, Muller-Scholze S, et al. The proatherogenic cytokine interleukin-18 is secreted by human adipocytes. Eur J Endocrinol 2005;152:863-8.
[14] Esposito K, Pontillo A, Ciotola M, et al. Weight loss reduces interleukin-18 levels in obese women. J Clin Endocrinol Metab 2002;87:3864-6.
[15] Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89:2548-56.
[16] Hung J, McQuillan BM, Chapman CM, et al. Elevated interleukin-18 levels are associated with the metabolic syndrome independent of obesity and insulin resistance. Arterioscler Thromb Vasc Biol 2005;25:1268-73.
[17] Netea MG, Joosten LA, Lewis E, et al. Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. Nat Med 2006;12:650-6.
[18] Miller GJ, Bauer KA, Barzegar S, et al. The effects of quality and timing of venepuncture on markers of blood coagulation in healthy middle-aged men. Thromb Haemost 1995;73:82-6.
[19] Miller GJ, Bauer KA, Barzegar S, et al. Increased activation of the haemostatic system in men at high risk of fatal coronary heart disease. Thromb Haemost 1996;75:767-71.
[20] Brull DJ, Montgomery HE, Sanders J, et al. Interleukin-6 gene −174G>C and −572G>C promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. Arterioscler Thromb Vasc Biol 2001;21:1458-63.
[21] Brull DJ, Sanders J, Rumley A, et al. Impact of angiotensin converting enzyme inhibition on post-coronary artery bypass interleukin 6 release. Heart 2002;87:252-5.
[22] Dhamrait SS, Stephens JW, Cooper JA, et al. Cardiovascular risk in healthy men and markers of oxidative stress in diabetic men are associated with common variation in the gene for uncoupling protein 2. Eur J Heart 2004;25:468-75.
[23] Stephens JW, Hurel SJ, Acharya J, et al. An interaction between the interleukin-6 −174G>C gene variant and urinary protein excretion influences plasma oxidative stress in subjects with type 2 diabetes. Cardiovasc Diabetol 2004;3:2.
[24] Alberti KG, Zimmet PZ. New diagnostic criteria and classification of diabetes—again? Diabet Med 1998;15:535-6.
[25] Stram DO, Haiman CA, Hirschhorn JN, et al. Choosing haplotype-tagging SNPS based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the Multiethnic Cohort Study. Hum Hered 2003;55:27-36.
[26] Day IN, Humphries SE. Electrophoresis for genotyping: microtiter array diagonal gel electrophoresis on horizontal polyacrylamide gels, hydrolink, or agarose. Anal Biochem 1994;222:389-95.
[27] Qin ZS, Niu T, Liu J.S. Partition-ligation-expectation-maximization algorithm for haplotype inference with single-nucleotide polymorphisms. Am J Hum Genet 2002;71:1242-7.

[28] Tregouet DA, Escolano S, Tiret L, et al. A new algorithm for haplotype-based association analysis: the stochastic-EM algorithm. Ann Hum Genet 2004;68:165-77.
[29] McCaskie PA, Carter KW, Palmer LJ. SimHap: a comprehensive modelling framework and a simulation-based approach to haplotypic analysis of population-based data. 2004.
[30] Diverse Populations Collaboration. Smoking, body weight, and CHD mortality in diverse populations. Prev Med 2004;38:834-40.
[31] Pickup JC, Mattock MB, Chusney GD, et al. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 1997;40:1286-92.
[32] Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care 2004;27:813-23.
[33] Biondi-Zoccai GG, Abbate A, Liuzzo G, et al. Atherothrombosis, inflammation, and diabetes. J Am Coll Cardiol 2003;41:1071-7.
[34] Mallat Z, Corbaz A, Scoazec A, et al. Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. Circ Res 2001;89:E41-5.
[35] Pradhan AD, Manson JE, Rifai N, et al. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001; 286:327-34.
[36] Woods SC, Gotoh K, Clegg DJ. Gender differences in the control of energy homeostasis. Exp Biol Med (Maywood) 2003;228:1175-80.
[37] Hansen E, Hajri T, Abumrad NN. Is all fat the same? The role of fat in the pathogenesis of the metabolic syndrome and type 2 diabetes mellitus. Surgery 2006;139:711-6.