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PAPER

A common polymorphism in the interleukin-6 gene promoter is associated with overweight

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OBJECTIVE: Human body fat mass is to a large extent genetically determined, but little is known about the susceptibility genes for common obesity. Interleukin-6 (IL-6) suppresses body fat mass in rodents, and IL-6 treatment increases energy expenditure in both rodents and humans. The −174 G/C single-nucleotide polymorphism (SNP) in the IL-6 gene promoter is common in many populations, and −174 C-containing promoters have been found to be weaker enhancers of transcription. Moreover, a SNP at position −572 in the IL-6 promoter has recently been reported to affect transcription. The objective was to investigate the association between the IL-6 gene promoter SNPs and obesity.
DESIGN: Trans-sectional association study of IL-6 gene promoter SNPs and indices of obesity.
SUBJECTS: Two study populations, the larger one consisting of hypertensive individuals (mean age 57 y, 73% males, $n=485$) and the other consisting of 20 y younger nonobese healthy females ($n=74$).
MEASUREMENTS: Genotyping for the −174 IL-6 G/C and the −572 G/C SNPs, body mass index (BMI), serum leptin levels, serum IL-6 levels, C-reactive protein, fasting blood glucose and various blood lipids.
RESULTS: The common −174 C allele ($f_C=0.46$), but not any −572 allele, was associated with higher BMI and higher serum leptin levels in both study populations. In the larger population, there were significant odds ratios for the association of CC (2.13) and GC (1.76) genotypes with overweight (BMI > 25 kg/m^2). Moreover, as the C allele was common, it accounted for a significant population-attributable risk of overweight (12%; CI 2–21%), although its average effect was modest in this sample.
CONCLUSION: Genetically determined individual differences in production of IL-6 may be relevant for the regulation of body fat mass.
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Keywords: interleukin-6; IL-6 promoter; polymorphism; leptin; body mass index

Introduction

Evidence for genetic control of human body weight and composition is well established, for instance, from adoption, twin and family studies.[1,2] So far single gene mutations have only been identified as the cause of morbid obesity in young individuals in about 5% of the cases.[1,3,4] Moreover, little is known about susceptibility genes contributing to modest obesity although knowledge is accumulating. One of the most successful routes for identification of body fat regulative mechanisms as well as genetic causes of obesity in humans has been systematic investigation of genes shown to affect energy metabolism in gene knockout mice.[5] Recent studies have shown that interleukin-6 (IL-6)-deficient mice develop mature onset obesity with high leptin levels in the circulation.[6] Moreover, intracerebroventricular IL-6 treatment decreases body fat and increases energy expenditure in rodents.[7,8] Thus, besides regulating the immune system,[9] IL-6 also plays a role in the regulation of body fat and energy expenditure.

Several single-nucleotide polymorphisms (SNPs) and repeat polymorphisms have been described in the IL-6 gene region, but there are few known polymorphisms of the coding region or of intron/exon boundaries of the IL-6

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gene.[10] The biallelic −174 IL-6 G/C SNP, which is located within the negative regulative domain of the IL-6 gene promoter, has been found to affect transcriptional regulation. Several studies of the biological relevance of the −174 G/C SNP have indicated that the most common −174 C-containing haplotypes are weaker enhancers of IL-6 transcription than those containing the −174 G.[11–14] Moreover, it has recently been reported that also the C allele of the −572 IL-6 G/C SNP is a stronger enhancer of IL-6 transcription.[15]

Based on these findings, we hypothesized that low IL-6 gene transcription, caused by the −174 C or the −572 G alleles, could contribute to obesity in humans. In the present study, we tested this hypothesis in two study populations to minimize the risk of coincidental findings.

Methods

Study subjects

Study population 1 was drawn from the Swedish part (7511 subjects) of the Captopril Prevention Project (CAPPP), a prospective randomized clinical trial conducted in Sweden and Finland during the 1990s. A total of 16 patients aged 25–66 y, with a measured diastolic blood pressure of 100 mmHg or more on two occasions, were randomly assigned captopril or conventional antihypertensive treatment (diuretics and/or β-blockers). Besides treatment for hypertension, the patients were on medication to be expected in this population, including treatment for anxiety, pain and depression. The exclusion criteria were secondary hypertension, serum creatinine concentration of more than 150 μmol/l and disorders that required treatment with β-blockers. Patients with diabetes were not excluded.[16] As the health status of the patients at the time of the blood sampling had not been recorded, C-reactive protein (CRP) was measured to avoid variability in blood chemistry due to ongoing inflammation.

Study population 2 was recruited by local advertisement at Huddinge hospital, Stockholm, and consisted of healthy women with a mean age 38 y. Altogether, 485 subjects of population 1 (73% males, mean age 57 y) and 74 subjects of population 2 (females, mean age 38 y) were included in this study. The clinical and biochemical characteristics of the subjects are represented in Table 1. The study was approved by the ethics committees of the University of Gothenburg and the Karolinska Institute.

Table 1 Clinical and biochemical characteristics of the study subjects

Subjects	*Population 1*	*Population 2*
Gender (M/F)	355/130	0174
Age (y)	56.8±0.31	38.3±1.24
BMI (kg/m^2)	27.5±0.18	23.9±0.31
SBP (mmHg)	173±0.84	114±1.91
DBP (mmHg)	106±0.32	72.2±1.23
Serum leptin (ng/ml)	21.4±1.08	12.3±0.81
Serum IL-6 (pg/ml)	2.36±0.19	NA
Fb-glucose (mM)	5.49±0.11	4.86±0.07
Total cholesterol (mM)	6.14±0.06	5.00±0.13
LDL (mM)	3.94±0.05	3.31±0.12
HDL (mM)	1.29±0.02	1.50±0.04
Triglycerides (mM)	2.02±0.05	1.07±0.08
CRP (ng/ml)	2.99±0.20	NA

SBP = systolic blood pressure, DBP = diastolic blood pressure, Fb-glucose = fasting blood glucose, CRP = C-reactive protein and NA = not analysed. Data are expressed as mean±s.e.m.

Study population 1

Biochemical analyses. Blood glucose was measured in morning samples from fasted subjects. Serum levels of IL-6 and leptin were determined by Quantikine High Sensitivity human IL-6 ELISA (R&D Systems, Minneapolis, MN, USA) and DSL-10-23100 Human Leptin ELISA (Diagnostic Systems Laboratories, Webster, TX, USA), respectively. Cholesterol and triglyceride levels were determined by fully enzymatic techniques on a Konelab 20 autoanalyzer (Thermo Clinical Labsystems, Espoo, Finland). High-density lipoprotein (HDL) was determined after precipitation of apolipoprotein (apo) B-containing lipoproteins with magnesium sulphate and dextrane sulphate (Thermo Clinical Labsystems). Low-density lipoprotein (LDL) was not measured directly, but was calculated from the following equation: LDL = Total cholesterol−HDL−triglycerides/5. CRP was measured by an ultrasensitive method (Orion Diagnostica, Espoo, Finland).

DNA isolation and genotyping. DNA was isolated using a commercial kit (Wizard® DNA Purification Kit, Promega, Madison, WI, USA). Amplification by PCR was performed on a Multiblock System (Hybaid, Middlesex, UK) according to the manufacturer's protocol (©Hybaid Limited March 2000 1.0.—Software Version 3.1). The forward and reverse primers for the −174 G/C IL-6 SNP were 5′-CACTTTCCCCCTAGTTGTGTCT and 5′-biotin-TTGTGCAATGTGACGTCCTTTAG, respectively, and the annealing temperature was 58°C. The forward and reverse primers for the −572 G/C IL-6 SNP were 5′-CCAGGCAGTCTACAACAGC and 5′-biotin-TGTTCTGGCTCTCCCTGTGA, respectively, and the annealing temperature was 52°C. The −174 G/C and the −572 G/C polymorphisms were genotyped using the dynamic allele-specific hybridization (DASH) method.[17] The following probes were used in the DASH analyses: 5′-GTGTCTTGCCATGCTAAAG for the C allele and 5′-GTGTCTTGCGATGCTAAAG for the G allele of −174 G/C, and 5′-ACAACAGCCCCTCACAGGG for the C allele and 5′-ACAACAGCCGCTCACAGG for the G allele of −572 G/C.

Study population 2

Biochemical analyses. Glucose, cholesterol, triglycerides and HDL were analysed by Huddinge University Hospital's routine chemistry laboratory. LDL was not measured directly, but was calculated from the following equation: LDL = Total cholesterol−HDL−triglycerides/5. Serum leptin levels were

analysed by Human Leptin radioimmunoassay kit (Linco, St Charles, MO, USA).

DNA genotyping. The genotyping was performed by PCR-RFLP as described[18] using primers 5′-TGACTTCAGCTT-TACTCTTTGT-3′ and 5′-CTGATTGGAAACCTTATTAAG-3′ and subsequent digestion of the PCR-product with SfaNI restriction enzyme.

Statistical methods

Data were analysed with the SPSS program (version 11.0.0; SPSS, Chicago, IL, USA). To compare the variables between the three different genotypes of −174 G/C SNP and −572 G/C SNP, respectively, we used the Kruskal–Wallis one-way (K–W one-way) ANOVA due to non-normally distributed data. χ^2 analysis was used for the investigation of the presence of Hardy–Weinberg equilibrium, and for overall comparison between overweight (body mass index (BMI) > 25 kg/m^2) and lean (BMI < 25 kg/m^2) subjects. Odds ratios for genotypes were calculated by logistic regression. Partial correlations were carried out to elucidate whether BMI or −174 G/C genotype was the strongest determinant of CRP levels in population.[1]

We calculated the population-attributable risk as described before.[19] The population-attributable risk = $(X-1)/X$, where $X=(1-f)^2+2f(1-f)r_1+f^2r_2$, f is the frequency of the risk allele ($f_C=0.47$) and r_1 and r_2 are the estimated genotype risk ratios of the GC (1.179; 95% CI 1.023–1.359) and CC (1.229; 95% CI 1.055–1.432) genotypes relative to GG ($r_1=(165/216)/(92/142)=1.179$ and $r_2=(90/113)/(92/142)=1.229$, see Table 2 for details). The confidence intervals (CIs) for the risk ratios were used to calculate the confidence interval for the population-attributable risk; the risk ratios (with CI limits) were assumed to have a linear relationship with the population-attributable risk.

To investigate the effects of −174 G/C genotype on serum leptin levels in women of populations 1 and 2, we used two-way ANOVA. Data of serum leptin levels were transformed with Blom's method in order to obtain normally distributed residuals in the two-way ANOVA.[20]

Differences of correlation coefficients (R) between the three −174 G/C genotypes, with respect to log IL-6/leptin ratios *vs* serum leptin levels, were formally tested after assuming normality.[21]

Investigation of −174 SNP together with the −572 SNP was carried out by creating allelic scores in a similar model as described in a recent publication.[15] An allelic score was determined by counting the number of alleles associated with enhanced IL-6 transcription, that is, the number of −572 C and −174 G alleles in each subject, giving a score of 0–4. Differences in indices of obesity and biochemical measurements were then analysed by K–W one-way ANOVA.

Data in text, figures or tables are given as mean ± standard error of the mean (s.e.m.). P-values of ≤0.05 were considered as significant.

Results

Study population 1

The frequencies of the −174 C and G alleles in this study population were 0.47 and 0.53, respectively. In all, 115 subjects (23.7%) had a CC genotype, 225 subjects (46.3%) a GC genotype and 145 subjects (29.9%) had a GG genotype. The genotypes were in Hardy–Weinberg equilibrium ($P=0.56$).

BMI and serum leptin levels were used as indices of obesity in both study populations. BMI in relation to the −174 G/C SNP in population 1 is shown in Figure 1. Individuals with the CC and GC genotypes had a mean BMI 5.2 and 3.7% higher, respectively, than the mean BMI of the GG genotypes ($P=0.003$ for the difference of genotypes). The overall comparison between overweight (BMI > 25 kg/m^2) and lean (BMI < 25 kg/m^2) study subjects of population 1 showed an association with −174 G/C genotype ($P=0.013$) and the odds ratios for individuals with GC and CC genotypes for being overweight were 1.76 (95% CI 1.10–2.80) and 2.13 (95% CI 1.20–3.77), respectively. Adjustments for age and gender changed the odds ratio to 1.71 (95% CI 1.04–2.80) for GC and 2.27 (95% CI 1.25–4.13) for CC genotypes (Table 2). Using risk ratio calculations and a recessive model, we could calculate the population-attributable risk of the −174 C allele in population 1. This risk was 12% (95% CI 2–21%), that is, the incidence of overweight in population 1 could be expected to decrease by 2–21% in the absence of the overweight-associated −174 C allele.

Table 2 Overall comparison of overweight and lean subjects in study population 1

		Overweight		*Lean*			*OR (95 % CI)*	
		n	%	n	%	P	*Univariate analysis*	*Multivariate analysis*
Genotype:	−174 GG	92	27	50	40		1.0	1.0
	−174 GC	165	48	51	41		1.76 (1.10–2.80)	1.71 (1.04–2.80)
	−174 CC	90	26	23	19	0.01	2.13 (1.20–3.77)	2.27 (1.25–4.132)

A BMI limit of 25 kg/m^2 was used to classify overweight or lean subjects in study population 1. The P-value is for overall comparison between overweight and lean subjects (χ^2 analysis). Odds ratios for genotypes were calculated by logistic regression analysis. Multivariate analysis was adjusted for age and gender.

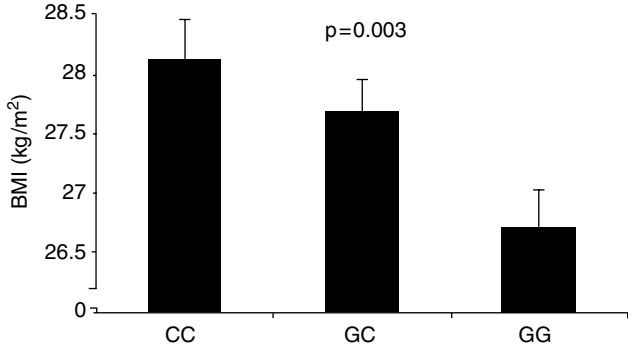

Figure 1 BMI of study population 1 according to the −174 G/C genotypes. Subjects with CC, GC and GG genotypes had mean BMIs of 28.1, 27.7 and 26.7 kg/m^2, respectively. The *P*-value is for comparison of the three genotypes (K–W one-way ANOVA). Data are shown as mean ± s.e.m.

To avoid interference from possible infection and/or inflammation, samples from subjects with a CRP level higher than 20 ng/ml were excluded from the association analysis of −174 G/C genotype and leptin, fasting blood glucose, total cholesterol, LDL, HDL, triglycerides, serum IL-6 as well as CRP levels. The serum leptin levels were more than two-fold higher in females than in males, confirming earlier reports.[22] Therefore, males and females were analysed separately (Figure 2). Mean serum leptin levels were 28.1 and 16.1% higher in males with CC and GC genotypes, respectively, than in males with the GG genotype ($P=0.002$ for the difference of genotypes, $n=333$). In females with the CC and GC genotypes, the mean serum leptin levels were 39.7 and 33.9% higher, respectively, than that of individuals with the GG genotype ($P=0.033$, $n=120$).

The frequencies of the −572 G and C alleles in this study population were 0.95 and 0.05, respectively. In all, 428 subjects (88.2%) had a GG genotype, 42 subjects (8.7%) a GC genotype and three subjects (0.6%) had a CC genotype. The genotypes were in Hardy–Weinberg equilibrium ($P=0.64$). The −572 G/C SNP did not, by itself or together with the −174 G/C SNP, affect any of the measured variables (data not shown). In line with previous results,[15] χ^2 analysis showed that there was a strong association between the −572 G/C SNP and the −174 G/C SNP ($P=0.001$).

The relation between −174 G/C genotype and various vital and blood chemistry parameters is shown in Table 3. The −174 G/C genotype was associated with CRP levels in serum. Individuals with the CC and GC genotypes had mean CRP levels that were 42.6 and 1.1% higher, respectively, than the mean CRP of the GG genotypes ($P=0.004$ for the difference of genotypes). This high CRP level in individuals carrying the –174 C allele associated with low IL-6 production may seem counterintuitive, given that IL-6 induces CRP, an acute-phase reactant.[9] However, this might be explained by the higher mean BMI in CC and GC genotypes. In this study, BMI was positively correlated with CRP level ($R=0.199$, $P<0.001$) in line with earlier reports,[23,24] and this was seen also when adjusting for −174 G/C genotype ($R=0.186$, $P<0.001$). In addition, the correlation between −174 G/C genotype and CRP level ($R=-0.109$, $P=0.02$) was not present after adjustment for BMI ($R=-0.09$, $P=0.06$).

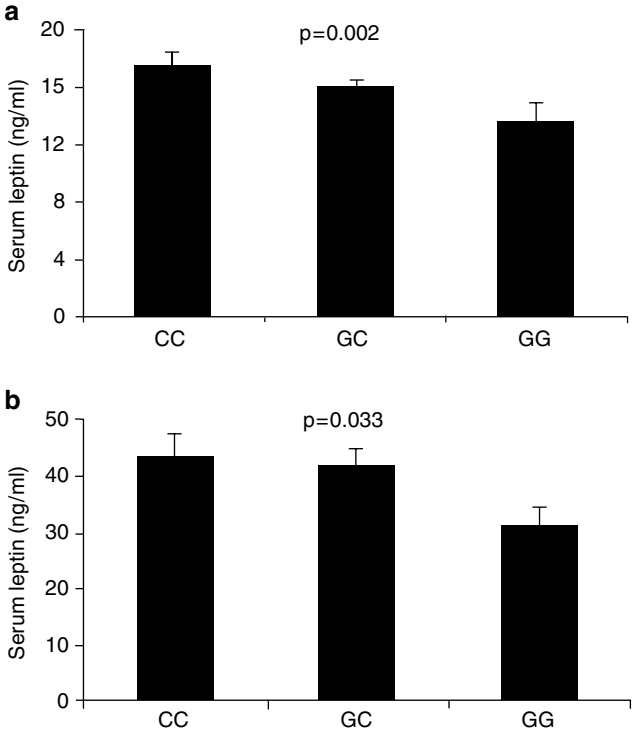

Figure 2 Serum leptin levels in male and female subjects according to the −174 G/C genotypes in study population 1. Serum leptin levels of study population 1 in male subjects (a) and in female subjects (b) plotted according to the −174 G/C genotype. The *P*-values are for comparison of the three genotypes (K–W one-way ANOVA). Data are shown as mean ± s.e.m. The scale of the *Y*-axes of panels a and b are different due to gender differences in leptin levels.

Table 3 Clinical and biochemical characteristics of study population 1 according to the –174 G/C genotype

	−174 IL-S genotype			
	CC	*GC*	*GG*	P-*value (K–W)*
Subjects (no./%)	115/23.7	225/46.3	145/29.9	
Age (y)	57.4 ± 0.58	56.4 ± 0.49	57.0 ± 0.54	0.61
SBP (mmHg)	174 ± 1.61	173 ± 1.27	172 ± 1.60	0.69
DBP (mmHg)	106 ± 0.63	107 ± 0.46	107 ± 0.63	0.66
Fb-glucose (mM)	5.64 ± 0.29	5.38 ± 0.15	5.53 ± 0.19	0.56
Total cholesterol (mM)	6.09 ± 0.14	6.17 ± 0.08	6.12 ± 0.11	0.97
LDL (mM)	3.92 ± 0.12	3.96 ± 0.07	3.92 ± 0.11	0.66
HDL (mM)	1.27 ± 0.05	1.28 ± 0.03	1.33 ± 0.05	0.20
Triglycerides (mM)	1.98 ± 0.09	2.09 ± 0.07	1.94 ± 0.10	0.22
IL-6 (pg/ml)	2.32 ± 0.29	2.14 ± 0.21	2.75 ± 0.48	0.45
CRP (ng/ml)	3.88 ± 0.60	2.75 ± 0.20	2.72 ± 0.40	0.004

The *P*-values are for comparison of the three genotypes (K–W one-way ANOVA). Data are shown as mean ± s.e.m.

The −174G/C genotype was not associated with differences in age, blood pressure, fasting blood glucose (fb-glucose), total cholesterol, LDL, HDL, or triglycerides (Table 3). Moreover, the −174 G/C SNP was not associated with prevalence or incidence of diabetes ($P=0.99$). Serum IL-6 levels were not affected by the IL-6 gene −174G/C polymorphism (Table 3). However, −174 C is associated with overweight as well as low IL-6 production, and serum IL-6 levels are positively correlated with fat mass.[25] Therefore, serum IL-6/leptin ratios were calculated in an attempt to relate serum IL-6 levels to body fat mass. These ratios were analysed in males and females separately due to the above-described gender difference in leptin levels. In males, GG genotypes had about two-fold higher serum IL-6/leptin ratio (0.39 ± 0.07) than both GC and CC genotypes (0.20 ± 0.03 and 0.19 ± 0.03, respectively; $P<0.003$ for the difference of genotypes). There was a similar, but not significant, trend in females (0.10 ± 0.02, 0.07 ± 0.01 and 0.07 ± 0.02 for GG, GC and CC genotypes, respectively; $P=0.28$ for the difference of genotypes). The logarithm of IL-6/leptin ratio was negatively correlated to BMI in males of all the three −174 IL-6 genotypes (CC: $R=-0.33$, $P=0.003$; GC: $R=-0.367$, $P<0.001$; GG: $R=-0.458$, $P<0.001$, but the R values did not differ between the genotypes; $P=0.6$). There was a similar tendency in females.

Study population 2

The frequencies of the −174 C and G alleles in this study population were 0.43 and 0.57, respectively. In all, 17 subjects (23.0%) had the CC genotype, 30 subjects (40.5%) the GC genotype and 27 (36.5%) had the GG genotype. The genotypes were in Hardy–Weinberg equilibrium ($P=0.57$). The relation between the −174 G/C genotype and BMI and serum leptin levels is shown in Table 4. Subjects with the CC and GC genotypes had mean BMIs 5.3 and 9.2% higher, respectively, than the mean BMI of the GG genotype ($P=0.03$). Compared to the GG genotype, serum leptin levels tended to be higher in subjects with CC and GC genotypes (66.8 and 26.4% higher, respectively), but the difference did not reach significance ($P=0.09$). However, there was a significant association between the −174 G/C genotype and serum leptin when women from populations 1 and 2 were analysed together in a two-way ANOVA ($P=0.023$ for the impact of −174 G/C). Thus, the −174 C allele seems to be associated with higher leptin levels, a marker of body fat, in both genders.

Generally, serum leptin levels were even lower in females in population 2 than in males in population 1. This might be due to the fact that these females had comparably low fat mass, as indicated by about 2.5 kg/m^2 lower BMI. Total cholesterol, LDL, HDL, triglycerides and b-glucose did not differ between the genotypes (data not shown).

Table 4 Age, BMI and serum leptin levels in study population 2 according to the –174 G/C genotype

	−174IL-6 genotype			
	CC	*GC*	*GG*	P-*value (K-W)*
Subjects (no./%)	17/23.0	30/40.5	27/36.5	
Age (y)	40.5 ± 2.49	36.9 ± 1.94	38.3 ± 2.15	0.53
BMI (kg/m^2)	24.0 ± 0.62	24.9 ± 0.49	22.8 ± 0.46	0.03
Serum leptin (ng/ml)	16.1 ± 2.42	12.2 ± 1.11	9.65 ± 0.75	0.09

P-values are for comparison of the three genotypes (K–W one-way ANOVA). Data are shown as mean$\pm$s.e.m.

Discussion

The results of this study supported our hypothesis that an IL-6 gene polymorphism, reported to be a weaker enhancer of IL-6 production, is associated with increased body fat mass and prevalence of overweight in humans. The C-containing genotypes of the common −174 G/C polymorphism of the IL-6 promoter were associated with increased indices of body fat mass. It should be kept in mind that we did not study randomized population samples, but on the other hand the results were seen in two separate study populations. The −174 C allele seems to be associated with lower IL-6 transcription in different cell systems.[11,12,14] There is also clear evidence from experimental studies that endogenous IL-6 suppresses body fat mass and prevents late-onset obesity.[6] We and others have also reported that IL-6 stimulates energy expenditure in both experimental animals[6,26] and in humans.[27–29] Moreover, endogenous IL-6 seems to influence energy expenditure during exercise.[30] Interestingly, the data of recent studies indicate that the –174 C allele is associated with decreased energy expenditure[31] and slightly decreased body temperature.[14] Taken together, these results indicate that the −174 C allele of the IL-6 promoter decreases IL-6 production, which in turn results in decreased energy expenditure and accumulation of body fat. As obesity is a risk factor for cardiovascular disease, it is of interest that the −174 C allele of the IL-6 gene has been associated with coronary heart disease.[10,32]

In this study, there was no correlation between serum IL-6 levels and the −174 G/C genotype. An association between the −174 C allele and low circulating levels of IL-6 has been shown in some[11,23,33,34] but not all[31,35] studies. There are several possible reasons that the association between the −174 G/C genotype and circulating IL-6 is variable. IL-6 in circulation originates from a diversity of cell types and tissues such as adipose tissue and immune cells[9,25,36] and there are also considerable irregular variations of circulating IL-6 during the day.[37] Alternatively, a genetically determined decrease in production of IL-6 per weight adipose tissue might theoretically cause increased obesity and thereby partly conceal the decrease in IL-6 levels in the blood circulation, in a similar way as reported for partial leptin

deficiency.[38] This possibility is supported by the findings that serum IL-6/leptin ratios, used as a surrogate marker for IL-6 production per adipose tissue mass, were found to be decreased in −174 C allele-containing genotypes.

The comparatively high serum IL-6 levels in obese individuals could implicate IL-6 resistance in a similar way as they are assumed to be leptin resistant.[39] However, there is evidence that the effects of IL-6 on body fat and energy expenditure are exerted at the level of the central nervous system (CNS) rather than the periphery.[6,7,26] Moreover, the levels of IL-6 in the CNS seem to be regulated in a different way than circulating IL-6 levels,[40] raising the possibility that circulating levels of IL-6 do not reflect the anti-obesity capacity of IL-6 in an individual.

Serum IL-6 levels (as well as inflammation in general) seem to be associated with obesity, insulin resistance and the metabolic syndrome in many human studies, as reviewed recently.[41] There are reports from the 1990s that treatment with high doses of IL-6 increases insulin resistance.[27,28] However, some more recent articles have not shown any increase in blood glucose after IL-6 treatment.[29,42] Moreover, recent results in mice indicate that lack of IL-6 or the IL-6 family signaling molecule STAT-3 also causes insulin resistance.[6,43] Interestingly, treatment with anti-IL-6 receptor antibodies have been shown to cause enhanced blood glucose levels in some patients with rheumatoid arthritis.[44] In summary, the effects of IL-6 seem complex and both decreased and increased IL-6 activity may cause increased insulin resistance. Such U-shaped dose–response curves are not uncommon in biology.

The determination of the IL-6 −174 G/C polymorphism in the present study was hypothesis driven. However, one shortcoming is that the samples were obtained from a study not originally designed for this purpose.[16] Moreover, the study population was not representative of a population in general, as it consisted predominantly of male subjects aged around 55–60 y with hypertension and a mean BMI in the overweight range. Therefore, it is of importance to confirm the results in an additional study population.[45] In the present study, the results were confirmed in healthy non-obese women that on average were 20 y younger, that is, a population that differed from the first one in several ways. The results of previous studies have shown conflicting results as −174 C allele has been associated with obesity in one,[46] but not in the other two[10,34] studies. However, our findings are supported by recent evidence that energy expenditure and body temperature are lower in the −174 CC genotype in healthy male and female subjects.[14,31] Altogether, this indicates that our finding is not coincidental although further studies are needed to investigate if these results can be applied to random samples of a population. It also remains to be investigated whether the effect of the −174 G/C genotype on overweight risk is confined to certain populations. There is evidence for considerable ethnic difference in both the −174 G/C polymorphism[11] and the causes of obesity,[1] and environmental factors including life style may also influence the effects of gene polymorphisms.[1,39]

The difference of 1.5 kg/m^2 in BMI and 4–5 kg in body weight between individuals with CC and GG genotypes at position −174 of the IL-6 gene promoter appears substantial, but could not by itself be the cause of severe obesity. In most cases, obesity is considered to be caused by multiple genetic variations in interplay with environmental factors.[1,39] The −174 C allele may contribute significantly to overweight and moderate obesity given that it is common, at least in European populations.[11] Indeed, our calculations support that the −174 C allele may account for a significant population-attributable risk of overweight although the confidence interval for this rough estimation was rather wide. This calculation is based on a −174 C allele frequency of 0.47, which is similar to other European populations.[10,11,18,34,35] However, as indicated in the studies discussed above,[10,34,46] the –174 C allele is probably not affecting body fat in all European populations, maybe due to interactions with yet unknown genetic and environmental factors.

In summary, we show that the −174 C allele of the IL-6 gene promoter is associated with overweight. The −174 C allele is common and has previously been associated with lower IL-6 gene transcription. Moreover, IL-6 has been shown to prevent obesity in rodents. Taken together, these data raise the possibility that −174 G/C SNP-regulated changes in the production of endogenous IL-6 contributes to the regulation of body fat mass, at least in some human populations.

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