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Interleukin-6 Genetic Variability and Adiposity: Associations in Two Prospective Cohorts and Systematic Review in 26,944 Individuals

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Context: IL-6 (*IL6*) is an immune-modulating cytokine associated with obesity in humans.

Objective: Our objective was to assess the associations between the genetic variability of *IL6* gene and adiposity and long-term changes.

Design and Subjects: We determined the linkage disequilibrium-tagging single-nucleotide polymorphisms of *IL6* gene in 2255 healthy women and 980 healthy men from two prospective cohorts. We also conducted a metaanalysis on the associations between polymorphism −174G>C (rs1800795) and adiposity.

Results: *IL6* haplotype 222211 (possessing rs2069827, rs1800797, rs1800795, rs1554606, rs2069861, and rs1818879; 1 codes the common and 2 codes the minor alleles) was consistently and significantly associated with greater waist circumference ($P = 0.009$ in men; $P = 0.0003$ in women) and baseline body mass index (BMI) ($P = 0.01$ in men; $P = 0.046$ in women) compared with the most common haplotype 111112. Haplotype 222211 was also associated with significantly higher early-adulthood BMI in women ($P = 0.007$). The haplotype-associated difference in BMI persisted significantly during the follow-up. A 5′ promoter polymorphism, rs2069827, was consistently associated with significantly higher early-adulthood BMI, baseline BMI, and waist circumference in men (carriers *vs.* noncarriers, $P = 0.01$, 0.007, and 0.008) and women ($P = 0.01$, 0.10, and 0.0016). The data from this study and a metaanalysis of 26,944 individuals did not support substantial relations between the best-studied polymorphism, −174G>C, and adiposity.

Conclusions: Our data from two independent cohorts indicate that the variability of the *IL6* gene is significantly associated with adiposity. Such associations are less likely to be caused by polymorphism −174G>C. **(*J Clin Endocrinol Metab* 92: 3618–3625, 2007)**

THE PREVALENCE OF obesity has been increasing alarmingly throughout the world and causes a multitude of comorbid conditions such as diabetes, cardiovascular disease, and cancer (1). The importance of genetic factors in determining susceptibility to obesity, in concert with environmental effects, has been well established (2). It is believed that many susceptibility genes may predispose to the common form of obesity, whereas the contribution of each gene is likely to be moderate.

In recent years, a growing body of evidence indicates that chronic low-grade activation of the immune system plays an important role in the etiology of obesity and related metabolic dysfunctions (3). Exploration of the genetic relationship between proinflammatory cytokine and adiposity has significant implications for understanding the pathogenesis of obesity. IL-6, a proinflammatory cytokine secreted by adipose tissue, immune cells, and muscles, can either accelerate or inhibit the inflammatory processes (4, 5). In humans, higher circulating IL-6 levels are associated with visceral fat and obesity (6, 7).

The associations between common variations in the *IL6* gene and adiposity have been examined in many studies (8–13). Most studies focused on a promoter polymorphism −174G>C (rs1800795) and generated conflicting results. Few studies examined the associations on the basis of replication and captured the comprehensive variance of the gene. Moreover, data on long-term changes in adiposity are sparse.

In the present study, we examined the associations of linkage disequilibrium (LD)-tagging single-nucleotide polymorphisms (SNPs) of the *IL6* gene (14), including polymorphism −174G>C, with adiposity and long-term changes in men and women from two independent, prospective cohorts. We also systematically review the associations between −174G>C and adiposity in a metaanalysis combining our data with previous studies.

Subjects and Methods

Study population

The Nurses' Health Study was established in 1976 when 121,700 female registered nurses aged 30–55 yr and residing in 11 large U.S. states completed a mailed questionnaire on their medical history and lifestyle. Information on lifestyle factors, including smoking, menopausal status and postmenopausal hormone therapy, body weight, and health and disease have been updated by validated self-administered questionnaires every 2 yr. A total of 32,826 women provided blood samples between 1989 and 1990. The Health Professional Follow-Up Study is a prospective cohort study of 51,529 American male health professionals aged 40–75 yr at study initiation in 1986 (15). Information about health and disease is assessed biennially by a self-administered questionnaire. Between 1993 and 1999, 18,159 men

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Abbreviations: BMI, Body mass index; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium; MET, metabolic equivalent task; SNP, single-nucleotide polymorphism.

TABLE 1. Characteristics of participants at cohort baseline

	Men	Women
No. of participants	980	2255
Age (yr)	56 (8)	44 (7)
BMI (kg/m^2)	25.0 (2.8)	23.8 (4.2)
Waist circumference (cm)	94.5 (8.6)	79.0 (11.2)
Physical activity, MET (h/wk)	22.1 (28.4)	4.1 (2.9)
Alcohol intake (g/d)	6.9 (0–101)	1.8 (0–112)
White (%)	96.2	96.0
Current smoker (%)	7.0	21.7
Post-menopausal (%)		19.6
Family history of diabetes (%)	13.7	22.5

Baseline is 1976 for women and 1986 for men; the categorical variables are presented as percentage and continuous variables are presented as mean (SD), except alcohol intake, which is presented as median (range).

provided blood samples. Participants in the present study were the healthy men and women from a diabetes study (14). We excluded those with missing body mass index (BMI) at cohort baseline. Finally, 2255 women and 980 men were included.

Assessment of adiposity and covariates

In 1986, participants were instructed to measure their waist at the level of the umbilicus and their hips at the largest circumference with a tape measure while standing relaxed and to report values to the nearest quarter inch. In 1987, the validity of self-reported waist and hip measures was assessed in a random sample of 140 participants living in the greater Boston area (16). The average of two technician measurements spaced 6 months apart was compared with the self-reported current weight and waist and hip circumference values on the most recent questionnaire. After adjustment for age and within-person variability, the Pearson correlation coefficients between the self-reported measures and the average of the two technician assessments were 0.95 for waist circumference, 0.88 for hip circumference, and 0.68 for waist-to-hip ratio. The correlations were essentially the

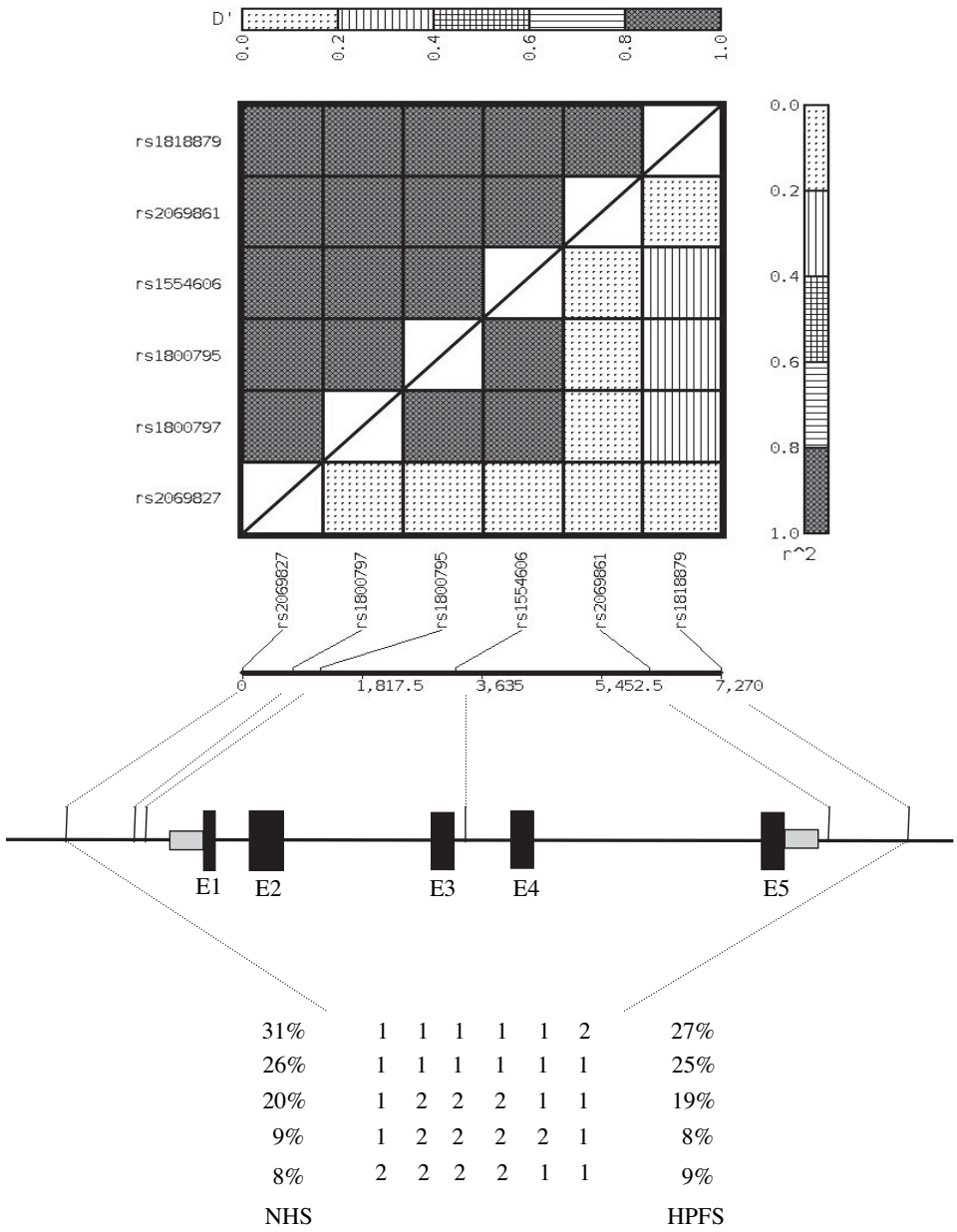

FIG. 1. The haplotype structure in the *IL6* gene locus. The six common polymorphisms from the *IL6* gene locus resulted in five common haplotypes. The haplotype frequencies were calculated for women and men separately. Polymorphism rs2069849, which is rare (<5%) and not used in haplotype inference, is not presented. HPFS, Health Professional Follow-up Study; NHS, Nurses' Health Study.

TABLE 2. Associations between *IL6* haplotypes and adiposity in men and women

Haplotypes	Frequency (%)	Young-adulthood BMI (kg/m^2)	*P* value	Cohort baseline BMI, kg/m^2	*P* value	Waist circumference (cm)	*P* value
Men							
111112	27	Reference		Reference		Reference	
111111	25	−0.01 (−0.29, 0.27)	0.94	−0.14 (−0.44, 0.17)	0.28	−0.15 (−1.24, 0.94)	0.77
122211	19	−0.02 (−0.35, 0.30)	0.88	−0.01 (−0.39, 0.36)	0.27	−0.38 (−1.62, 0.86)	0.55
122221	8	−0.17 (−0.66, 0.32)	0.48	0.09 (−0.41, 0.59)	0.95	0.05 (−1.57, 1.68)	0.96
222211	9	0.37 (−0.08, 0.82)	0.10	0.52 (0.11, 0.93)	0.01	1.96 (0.48, 3.40)	0.009
Women							
111112	31	Reference		Reference		Reference	
111111	26	0.05 (−0.20, 0.31)	0.68	0.24 (−0.10, 0.58)	0.17	0.56 (−0.48, 1.63)	0.29
122211	20	0.004 (−0.26, 0.97)	0.97	−0.17 (−0.55, 0.21)	0.37	−0.51 (−1.60, 0.61)	0.38
122221	9	−0.23 (−0.58, 0.12)	0.19	−0.36 (−0.86, 0.13)	0.14	−0.99 (−2.54, 0.56)	0.21
222211	8	0.47 (0.13−0.82)	0.007	0.54 (0.01, 1.06)	0.046	2.51 (1.14, 3.89)	0.0003
Men and women							
222211 *vs.* 111112		0.43 (0.16, 0.71)	0.002	0.53 (0.20, 0.85)	0.001	2.26 (1.24, 3.25)	<0.0001

For the haplotype coding, 1 represents the common allele and 2 represents the minor allele. The haplotypes possess six polymorphisms (from left to right) including rs2069827, rs1800797, rs1800795, rs1554606, rs2069861, and rs1818879.

same for all strata of age, smoking status, and BMI, which was calculated as weight in kilograms divided by the square of height in meters. Both waist circumference and waist-to-hip ratio have been used as measures of central obesity in epidemiological studies (17). In the present study, we used waist circumference as the primary measure because of its better correlation with the technician measurements in the validation study than waist-to-hip ratio. Physical activity was expressed as metabolic equivalent task (MET)-hours based on self-reported types and durations of activities over the previous year.

SNP selection and genotype determination

DNA was extracted from the buffy coat fraction of centrifuged blood using the QIAmp Blood Kit (QIAGEN, Chatsworth, CA). We selected tagging SNPs from the SeattleSNPs database that uses a clustering approach (ldSelect program) to bin SNPs with similar r^2 for one threshold (0.64) (18) (http://droog.gs.washington.edu/ldSelect.html). One tag SNP was chosen for each cluster bin (frequency >5%) giving a priority to those located in the coding region, 5′ promoter, and 3′-untranslated region. We also included reported polymorphisms that were previously associated with adiposity. Seven common polymorphisms (rs2069827, rs1800797, rs1800795, rs1554606, rs2069849, rs2069861, and rs1818879) were genotyped using TaqMan SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA). Replicate quality control samples were included and genotyped with more than 99% concordance. Successful rate of genotyping ranged from 94–98%.

Metaanalysis of the association between −174G>C (rs1800795) and adiposity

Clinical studies in which the *IL6* polymorphism −174G>C had been related to adulthood adiposity (BMI, waist circumference, and waist-to-hip ratio) were identified by electronic searches of the MEDLINE (PubMed) and Online Mendelian Inheritance in Man (OMIM) databases (through April 2007) and hand-searching of reference lists of obtained articles. We did not include the studies conducted exclusively in patients with apparent diseases, including diabetes, cancer, and cardiovascular diseases, and the studies in children. A total of 18 studies with available data on BMI/waist-to-hip ratio by *IL6* genotypes (reported or obtained by personal communication with the authors) were identified. Because there was no strong biological plausibility that specific genetic inheritance mode may be more appropriate, we decided to use an analysis considering all genotypes separately. We assessed the significance of between-study heterogeneity with the Q statistic.

To account for heterogeneity among studies, the pooled estimates of mean difference between genotypes were calculated using of random-effects metaanalysis by the method of DerSimonian and Laird (19) with inverse-variance (SE) weighting. Because *IL6* genotype deviated from Hardy-Weinberg equilibrium (HWE) in some studies (8, 20), we performed sensitivity analyses by excluding these observations. To assess publication bias, a funnel plot of genetic difference in anthropometric outcomes *vs.* SE was visually inspected (21). We used metaregression to test for heterogeneity of the pooled associations by sex, age, and covariate adjustment. Metaanalyses were performed with Stata (version 8.2; Stata Corp., College Station, TX).

Statistical analyses

A χ^2 test was used to assess whether the genotypes were in HWE. The geometric means of adiposity measures (BMI and waist circumference) were compared among the genotype groups using general linear models, adjusting for age (continuous). In the multivariate analyses, we also adjusted for physical activity (<1.5, 1.5–5.9, 6.0–11.9, 12–20.9, and ≥21.0 MET h/wk), smoking (never, past, and current), and menopausal status [pre- or postmenopausal (never,

TABLE 3. BMI and waist circumference according to rs2069827 genotypes

	Men				Women				*P*, all
	GG	GT	TT	*P* value	GG	GT	TT	*P* value	
Early-adulthood BMI (kg/m^2)[a]	22.6 (778)	23.2 (154)	22.4 (10)	0.01	21.4 (1727)	21.8 (335)	22.0 (16)	0.01	<0.001
Baseline BMI (kg/m^2)[b]									
All subjects	24.9 (796)	25.5 (159)	26.4 (10)	0.007	23.8 (1832)	24.1 (353)	25.4 (18)	0.10	0.017
Nonsmokers	24.9 (735)	25.5 (155)	26.6 (9)	0.004	23.9 (1420)	24.3 (280)	25.9 (14)	0.06	0.019
Waist circumference (cm)[c]									
All subjects	94.2 (689)	96.3 (139)	98.0 (8)	0.008	78.7 (1260)	80.8 (240)	86.6 (13)	0.0016	<0.0001
Nonsmokers	94.0 (636)	96.3 (136)	96.2 (8)	0.004	78.7 (1118)	81.3 (205)	87.9 (12)	0.0002	<0.0001

P values are for comparisons between carriers and noncarriers of the polymorphism; tests for sex heterogeneity were not significant.
[a] Women, 18 yr; men, 21 yr.
[b] Women, 1976; men, 1986; age adjusted.
[c] Women, 1986; men, 1987; age adjusted.

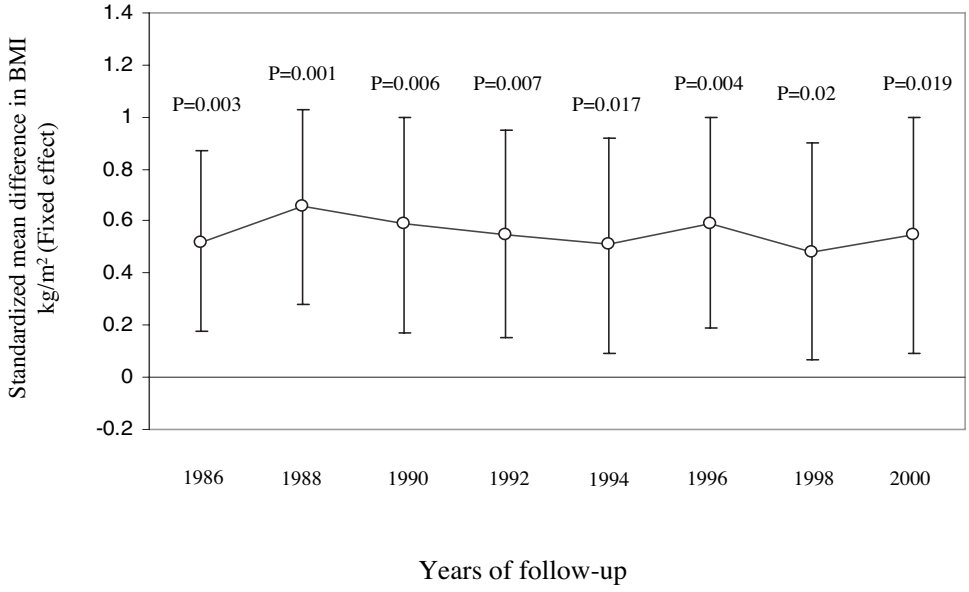

FIG. 2. Haplotype difference in BMI between haplotype 222211 and 111112 (reference) in the pooled sample of men and women during follow-up from 1986–2000. Haplotypes possess polymorphisms rs2069827, rs1800797, rs1800795, rs1554606, rs2069861, and rs1818879; 1 codes the common and 2 codes the minor allele. The standardized mean difference, 95% confidence interval, and *P* values for comparisons are presented.

past, or current hormone use); for women only]. Haplotype analysis was conducted based on the stochastic-EM algorithm using the THESIAS program (22). We calculated least-square means for changes in BMI from baseline (1976 for women and 1986 for men) through 2000 across genotypes, adjusting for baseline age and BMI. The SAS statistical package was used for the analyses (SAS, version 8.2 for UNIX). All *P* values are two sided.

Results

Associations between IL6 variations with adiposity in Nurses' Health Study and Health Professional Follow-Up Study

Selected baseline characteristics of the participants are summarized in Table 1. Women were younger and leaner but engaged in less physical activity than men. Seven LD-tagging SNPs for the *IL6* gene were genotyped (rs2069827, rs1800797, rs1800795, rs1554606, rs2069849, rs2069861, and rs1818879). None of these polymorphisms significantly deviated from HWE in the study samples. All the polymorphisms were in strong LD ($D' > 0.95$ and r^2 ranging from 0.01–0.92; Fig. 1).

Because the polymorphisms included in the present study were primarily identified for tagging purposes, we first examined the associations between the inferred haplotypes and adiposity measures. Polymorphism rs2069849 was rare (<5%) in both study populations and did not substantially contribute to the haplotype variance. Therefore, the haplotypes were inferred from the other six polymorphisms (Fig. 1). Using the haplotype-based general linear model, statistically significant differences were consistently observed between haplotype 222211 and the most common haplotype 111112 in baseline BMI (men at 1987 and women at 1986) and waist circumference (women at 1986 and men at 1987) in men and women, adjusting for age (Table 2). Further adjustment for other covariates did not appreciably change the results. Haplotype 222211 was also significantly associated with early-adulthood BMI in men (21 yr old) and marginally associated with early-adulthood BMI in women (18 yr old). In the pooled sample of men and women, the haplotype-associated difference became more significant.

Among the tagging SNPs, rs2069827 was consistently associated with adiposity measures in both genders (Table 3), in whom the early-adulthood BMI and waist circumference were significantly higher in carriers of allele T compared with the noncarriers. Restricting the analyses to the nonsmokers gave rise to similar results. In addition, rs2069827 was also significantly associated with baseline BMI in men and marginally related to baseline BMI in women. No other polymorphisms showed significant and consistent associations with the adiposity (data not shown).

We also examined the associations between *IL6* haplotypes and BMI during the follow-up. To improve the study power, we pooled the data from men and women for the overlapping follow-up years (1986, 1988, 1990, 1992, 1994, 1996, 1998, and 2000; Fig. 2). Haplotype 222211 was significantly associated with 0.48–0.66 kg/m^2 higher BMI compared with the most common haplotype during the follow-up. Our analyses indicated that *IL6* genotypes, individually or in haplotype, were not significantly associated with the longitudinal changes in BMI from early adulthood to baseline and during the follow-up in both men (1986–2000) and women (1976–2000) (data not shown).

Metaanalysis of the associations between polymorphism −174G>C (rs1800795) and adiposity in 26,944 subjects

In total, 19 studies (25 observations) of 26,944 participants, including the present study, were included in the metaanalysis (8, 9, 11, 20, 23–34). Six studies reported the associations separately for men and women (24, 31, 33), for lean and obese subjects (9), or for young and old subjects (25). We treated the results from each subpopulation as independent observations. For studies reporting the adiposity by genotypes in patients with diseases such as

coronary heart disease and hypertension and healthy controls (27, 29), we used the data from the controls only. The results of individual studies and the pooled estimate are presented in Fig. 3. In the analysis including all studies, the summary standardized mean differences in BMI between genotypes, GC *vs.* GG and CC *vs.* GG, were not statistically significant. Between-study heterogeneity was evident ($P < 0.05$).

The pooled estimate was not appreciably changed in sensitivity analyses by removing studies with deviation of the genotype distribution from HWE (8, 20) and by exclusion of the studies in obese samples (9, 23). Little evidence for publication bias was present (data not shown). In metaregression analyses, we did not observe significant effect modification by sex, age (55 yr old as cutoff), and adjustment for covariates (yes or no). Summary estimates of genotypic difference in BMI were comparable across strata by these characteristics and were not statistically significant in any of the strata (Table 4). Some studies (23, 28, 33) presented the C as the common and G the minor

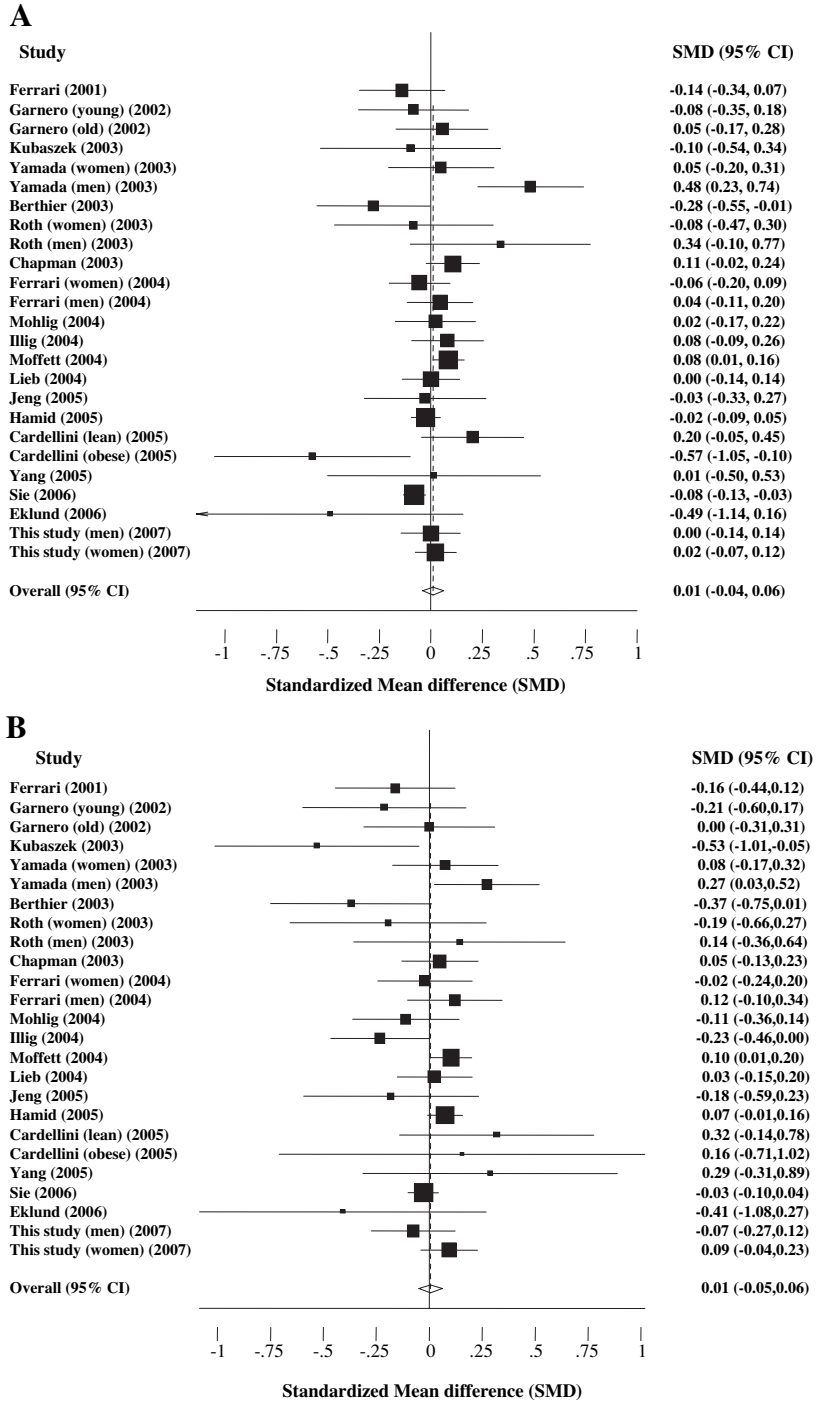

FIG. 3. Metaanalysis of studies on *IL6* polymorphisms −174G>C (rs1800795) and BMI. A, GC *vs.* GG; B, CC *vs.* GG. Size of *squares* indicating mean values is proportional to the weight that each study contributed to the aggregated estimates. Summary standardized mean differences with 95% confidence intervals (CI) are marked by *diamonds*.

TABLE 4. Metaanalysis of *IL6* −174 G>C and BMI by study characteristics

	No. of studies	GC *vs.* GG			CC *vs.* GG		
		SMD in BMI (95% CI)	*P*	*P* for heterogeneity	SMD in BMI (95% CI)	*P*	*P* for heterogeneity
Gender							
Men	7	0.05 (−0.14, 0.24)	0.61	0.002	0.05 (−0.01, 0.12)	0.11	0.42
Women	8	0.03 (−0.02, 0.08)	0.26	0.44	0.02 (−0.15, 0.20)	0.79	0.06
Mixed	10	0.00 (−0.07, 0.06)	0.95	0.04	0.00 (−0.06, 0.06)	0.97	0.26
Age							
<55 yr	15	−0.02 (−0.08, 0.04)	0.50	0.03	0.02 (−0.04, 0.08)	0.50	0.31
≥55 yr	10	0.05 (−0.02, 0.12)	0.19	0.04	0.01 (−0.08, 0.10)	0.84	0.07
Adjustment for covariates							
Yes	8	0.00 (−0.06, 0.05)	0.89	0.34	−0.02 (−0.12, 0.08)	0.72	0.07
No	17	0.02 (−0.06, 0.09)	0.65	0.01	0.03 (−0.03, 0.09)	0.36	0.29

CI, Confidence interval; SMD, standardized mean difference.

allele. This may be due to either genotyping on different strands of the chromosome or the ethnic differences (Asian *vs.* Caucasian populations). Removing these studies or reversing the allele coding did not considerably change the results.

In addition, we did not find significant differences in waist-to-hip ratio (9, 11, 28, 35) or waist circumference (8, 26) between −174G>C genotypes in the metaanalyses (data not shown).

Discussion

We have examined the associations between LD-tagging SNPs of *IL6* gene in relation to adiposity and longitudinal changes. A haplotype 222211 (possessing rs2069827, rs1800797, rs1800795, rs1554606, rs2069861, and rs1818879; 1 codes the common allele and 2 codes the minor allele) was consistently and significantly associated with higher waist circumference and BMI in two independent cohorts of men and women. The *IL6* genotype-associated difference in adiposity persisted throughout adulthood.

The present findings are in line with the known biological roles of IL-6 in modulating body fat and obesity (6, 7, 36–38). Several studies have assessed the associations between the common variations in the *IL6* gene, especially those at the 5′ promoter region, and adiposity in humans. The best-studied polymorphism −174G>C was associated with BMI and central adiposity in some (8, 12) but not other studies (29, 39). However, this polymorphism was not associated with adiposity in the present study or the metaanalyses. Functional analyses suggest that the −174C allele may lower gene expression (40) and *in vivo* inflammatory response (41). Nevertheless, studies on the relation between −174G>C and circulating IL-6 generated conflicting results (14, 34, 28, 42, 43). Of note, recent large-scale studies and metaanalysis indicate that −174G>C may not have substantial effects on the risk of coronary heart disease (32) or type 2 diabetes (14). Interestingly, polymorphism rs2069827, which resides in the 5′ promoter and is in strong LD with −174G>C, was consistently associated with adiposity in the present study among both men and women.

It has been suggested that more than one polymorphic site in the *IL6* gene may be functional and influence gene transcription probably through complex interactions (44). Our observation of the haplotype associations suggests that the combination of several variations in the *IL6* gene, which likely occur in the haplotype-driven selection (45), may contribute jointly to the elevated adiposity. Such a cooperative action of multiple alleles was also observed in relation to other clinical outcomes (46).

The associations between *IL6* variations and waist circumference are important because visceral fat is more pathogenic for the metabolic disorders than the other fat depots (47). Nevertheless, the metabolic changes solely related to *IL6* variants may be insufficient to lead to transition from subclinical to clinical diseases such as coronary heart disease and type 2 diabetes. Our data indicate that the effects of genetic variations of *IL6* on adiposity may exhibit since an early stage of life. This is consistent with the observations that the *IL6* polymorphism was associated with body composition in children (48). *IL6* variations were not associated with the long-term changes in adiposity during adulthood. Even so, we could not exclude the possibility that individuals with higher BMI are more likely to change their diets and lifestyle than those with normal BMI (49, 50), which could have modulated the strength of the genetic associations.

A principle challenge in genetic association studies has been the high rate of false-positive results. It is widely accepted that study design based on replication validity, as compared with statistical correction, is a better way to limit type I error rates and provide reliable confirmation of genetic associations (51, 52). As a major strength, the present study was conducted in two independent cohorts. The associations between *IL6* haplotypes and adiposity were highly consistent in the study populations, suggesting that our findings are less likely due purely to chance. Population stratification may bias the observed associations. However, our populations are racially homogeneous, with the majority of the participants being white (~96%). Further adjustment for ethnicity or removing those of minority ethnicity from the analyses did not change the associations.

In conclusion, we have found *IL6* variations were significantly associated with BMI and waist circumference. The differences in adiposity associated with *IL6* genotypes/haplotypes were modest. Given the temporal relationship between genetic variants and adiposity phenotypes, our findings suggest that the genotype-associated

changes in IL-6 are causally involved in the development of obesity. Additional analyses are warranted to replicate the associations in other populations, to identify the potential causal variants, and to elucidate related functional changes that may affect body fatness.

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L.Q., C.Z., R.M.v.D., and F.B.H. have nothing to declare.

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