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The *FTO* rs9939609 polymorphism is associated with short leukocyte telomere length in nonobese individuals

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Abstract

The fat mass and obesity-associated (FTO) rs9939609 polymorphism have been associated with the increased metabolic risk and mortality, irrespective of obesity. The mechanism underlying this association is not known. We aimed to evaluate whether the FTO rs9939609 risk variant is independently associated with metabolic risk factors and/or leukocyte telomere length (LTL). We further aimed to investigate whether this relationship is modified by obesity status.

A total of 2133 participants were recruited from the Korean Genome and Epidemiology Study. LTL was measured using the real-time quantitative polymerase chain reaction methodology. The *FTO* rs9939609 polymorphism was genotyped using DNA samples collected at baseline.

The proportions of the TT, TA, and AA genotypes were 76.7%, 21.5%, and 1.8%, respectively, and obese subjects comprised 44.5% of the total subjects. Among the 1184 nonobese subjects, body mass index, waist circumference, and visceral fat area were higher in subjects with the *FTO* risk allele than in noncarriers. In contrast, only high-sensitive C-reactive protein level was associated with the *FTO* risk allele in the obese subjects. LTL was significantly shorter in carriers of the *FTO* risk allele compared with noncarriers after controlling for several confounding factors ($P < .01$). Of particular note, this significant association between the *FTO* risk allele and LTL appeared only in nonobese subjects ($P = .03$). Multivariate linear regression analyses identified older age, low high-density lipoprotein cholesterol level, and the presence of the *FTO* risk allele as independent risk factors affecting LTL. This finding was evident only in nonobese subjects.

The *FTO* rs9939609 polymorphism is an independent risk factor for obesity and also for biological aging in the nonobese population.

Abbreviations: FTO = fat mass and obesity-associated, LTL = leukocyte telomere length.

Keywords: *FTO* polymorphism, leukocyte telomere length, obesity

1. Introduction

The etiology of obesity is influenced by genetic background as well as environmental factors. One of the genes associated with common obesity is the fat mass and obesity-associated (*FTO*) gene. In 2007, 3 independent genome-wide association studies (GWAS) demonstrated a strong association between genetic variance within *FTO* and human obesity.[1–3] This association has been reproduced in multiple populations, including Asians

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and different age groups.[4] In addition to its association with obesity, *FTO* variants are well known to be associated with increased risk of type 2 diabetes, hypertension, heart failure, dyslipidemia, metabolic syndrome, cardiovascular disease (CVD), and cancers.[5–8] Furthermore, the *FTO* variant has been shown to increase the risk of mortality, independent of fatness.[9,10] Although many studies have focused on *FTO*, the mechanism by which *FTO* variants are linked to metabolic abnormalities independent of obesity remains unclear.

Telomeres consist of tandem TTAGGG repeats that cap chromosome ends and prevent genomic instability.[11] Telomere shortening is widely accepted as a biomarker for aging and stress-related conditions.[12] Short leukocyte telomere length (LTL) has been associated with a variety of age-related disorders, including obesity, type 2 diabetes mellitus, CVD, and certain types of cancers.[13–16] Since the *FTO* gene itself is related to several age-related disorders, it is possible that *FTO* is also associated with telomere shortening independent of obesity. In other words, carriers of *FTO* variants may have short telomeres, resulting in chromosomal instability, an increased risk of CVD, and even premature death. Indeed, Dlouha et al[17] reported that LTL was significantly shorter in carriers of *FTO* variant (AA) in post-menopausal women, suggesting a potential mechanism to explain the association between *FTO* and the increased risk of chronic disease and mortality.

Obesity is a major public health problem that is associated with aging and short longevity. However, not all individuals with obesity have an increased risk of mortality compared with nonobese individuals. For example, metabolically obese normal weight (MONW) subjects can be at higher risk of CVD or death than metabolically healthy obese (MHO) subjects.[18] This phenomenon is more prominent in Asians, who tend to have a lower body mass index (BMI) than Europeans. In addition, *FTO* variants are low frequency, and their effect size is smaller in Asians.[19] Therefore, the impact of *FTO* on obesity and metabolic risk factors may differ according to ethnicity and/or obesity status. Similarly, the relation between *FTO* and telomere length maybe different in a given population compared to those that have been previously studied. However, few studies have investigated the relevance of *FTO* variants to telomere attrition.

Therefore, the aim of this study was to evaluate whether *FTO* variants are independently associated with metabolic risk factors and/or LTL. We also aimed to determine whether this relationship varies according to obesity status.

2. Methods

2.1. Subjects

All study subjects were derived from the Ansan cohort of the Korean Genome Epidemiology Study (KoGES), an ongoing population-based cohort study that began in 2001. Participants in the KoGES have been biennially evaluated for demographics, lifestyle characteristics, sleep-related factors, anthropometric and biochemical variables, and health status (including medical illness and medications). All information was collected by trained interviewers. Initially, the original cohort consisted of 5020 participants between 2001 and 2002. During the 10-year follow-up period, 3052 (60.8%) were followed up in the 6th KoGES between 2011 and 2012. Data from the sixth biennial examination were used for the current study because measurements of telomere length were taken at that time. Only visceral fat-related data were selected from the fifth examination, when abdominal computed tomography (CT) was performed. Among 3052 participants in the 6th KoGES, a total of 2314 subjects measured their telomere length. After excluding individuals whose genetic data were unavailable (n = 181), 2133 participants were eligible for analysis. Each participant signed an informed consent form. This study was performed according to the principles of the Declaration of Helsinki of the World Medical Association and was approved by the institutional review board of Korea University Ansan Hospital.

2.2. Assessments

2.2.1. Demographic, anthropometric, and laboratory measurements. All participants responded to an interviewer-administered questionnaire and underwent a comprehensive physical examination. Lifestyle characteristics of smoking status and alcohol consumption were categorized as never, former, and current. The exercise level during the previous month was categorized as never, light (1–3 times/week, ≥30 min/session), or regular (≥3 times/week, ≥30 min/session). Hypertension was diagnosed when systolic blood pressure (SBP) or diastolic blood pressure (DBP) was equal to or above 140 or 90 mm Hg, respectively, or when participants took antihypertensive medications. Height was measured to the nearest 0.1 cm using a fixed wall-scale measuring device. Weight was measured to the nearest 0.1 kg using an electronic scale that was calibrated before each measurement. BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured to the nearest 0.5 cm in a horizontal plane at the level of the umbilicus at the end of a normal expiration.

Blood was drawn for biochemical analysis after an overnight fast. Plasma glucose, serum triglyceride, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol levels were measured with an autoanalyzer (ADVIA 1650; Siemens, Tarrytown, NY). High-sensitive C-reactive protein (hsCRP) level was measured by an immunoassay (ADVIA1800, Siemens). The insulin level was measured with an immunoradiometric assay kit (INS-IRMA Kit; BioSource, Nivelles, Belgium) using a Packard γ counter system. Participants also underwent a 75-g oral glucose tolerance test.

2.2.2. Measurements of LTL. Relative LTL was measured using the quantitative polymerase chain reaction as previously reported.[20] Briefly, leukocyte genomic DNA was extracted with the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) from peripheral blood samples. Purified DNA samples were diluted and quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). To determine the relative LTL, the ratio of the telomere repeat copy number to the copy number of the single-copy *36B4* gene, which encodes acidic ribosomal phosphoprotein, was determined using an iQ Multi-Color Real-Time Polymerase Chain Reaction Detection System (Bio-Rad, Hercules, CA). The final concentrations of the polymerase chain reaction reagents were: 1 × SYBR Green SuperMix (Bio-Rad); 50 ng of DNA; 0.2 μM of telomere primers (forward: 5′-GGTTTTTGAGGGTGAGGGT-GAGG GTGAGGGTGAGGGT-3′; reverse: 5′-TCCCGAC-TATCCC TATCCCTATCCCTATCCCTATCCC TA-3′); and 0.3 μM of *36B4* primers(forward: 5′-CAGCAAGT GGGAAGGTGTAATCC-3′; reverse: 5′-CCCATTCTATCAT-CAACGGGTACAA-3′). Reactions were performed using telomere and *36B4* primer sets in a single 96-well plate. Each plate included a reference DNA sample. A 4-point standard curve was

established to transform cycle thresholds into units of nanograms of DNA. A validity test showed that the Pearson correlation coefficients were 0.78 (intra-assay) and 0.69 (inter-assay) when 25 samples were run in triplicate.

2.2.3. FTO rs9939609 polymorphism genotyping. Genotyping of the *FTO* rs9939609 polymorphism was carried out as part of an earlier GWAS.[21] FTO rs9939609 is an SNP located in the first intron of the FTO gene on chromosome 16.[1] Detailed information on genomic sample preparation, genotyping methodology, and quality control is available in.[21] Briefly, blood samples were collected for genotyping and biochemical analyses from participants who had fasted for at least 8 hours. Genomic DNA was harvested from blood samples, and 500 ng of DNA was processed using an Affymetrix Genome-wide Human SNP Array 5.0 (Affymetrix, Inc., Santa Clara, CA). All genotyping call rates were greater than 95%, and the genotype distribution of the rs9939609 alleles was in Hardy–Weinberg equilibrium (HWE) ($P=.31$).

2.2.4. Definitions of diabetes mellitus, metabolic syndrome, cardiovascular disease, and obesity. Diabetes mellitus was diagnosed when fasting plasma glucose (FPG) was ≥7.0 mmol/L, or the 2-hour plasma glucose was ≥11.1 mmol/L after the 75-g oral glucose tolerance test, or participants took anti-diabetic medication.[22] Insulin resistance was estimated with the homeostasis model of assessment for insulin resistance (HOMA-IR) and calculated as fasting glucose (mmol/L) × fasting insulin (μU/mL) / 22.5.[23] According to the guidelines of the National Cholesterol Education Program Adult Treatment Panel III,[24] metabolic syndrome was defined by the presence of 3 or more of the following 5 conditions: (i) abdominal obesity (waist circumference ≥90 cm for men and ≥85 cm for women); (ii) hypertriglyceridemia (fasting plasma triglycerides ≥150 mg/dL); (iii) low high-density lipoprotein cholesterol (<40 mg/dL in men and <50 mg/dL in women); (iv) hypertension (SBP ≥130 mm Hg or DBP ≥85 mm Hg, or taking antihypertensive medications); and (v) hyperglycemia (FPG ≥100 mg/dL or taking antidiabetic medications). Subjects with documented events or medical records of myocardial infarction, angina, heart failure, stroke, or peripheral artery disease were considered to have CVD. Obesity was defined as BMI ≥25 kg/m^2, according to the Asian-specific BMI cut-off from the World Health Organization.[25]

2.2.5. Visceral fat measurements. The abdominal adipose tissue area was quantified using single-slice CT. For measurements, a Brilliance 64 CT scanner (Philips, Cleveland, OH) was used with a 120-kV exposure. A 5-mm CT slice scan was acquired at the L4–L5 vertebral interspace, and images were converted into a compatible format using a commercial software program (EBW, Philips). The total area of intra-abdominal fat was delineated by manual tracing within the muscle wall, and the visceral fat area (VFA) was defined as the area with an attenuation range between –190 and –30 Hounsfield units.

2.2.6. Statistical analysis. Baseline characteristics were compared among groups stratified by the presence or absence of the *FTO* risk allele and obesity status by Student's *t* test for numeric variables and the chi-square test for categorical variables. Non-normally distributed variables, such as HbA1c, HOMA-IR, triglyceride, insulin, and hsCRP, were expressed as the median and interquartile range for each group, and the differences were tested for statistical significance after logarithmic transformation. We also compared telomere length between groups stratified by *FTO* allele and obesity status using analysis of covariance after adjusting for age, sex, BMI, smoking, alcohol, exercise, DM, CVD, and HTN status. To examine the relationships between clinical parameters and LTL, Pearson correlation analysis was performed. Stepwise multivariate linear regression analyses were conducted to evaluate independent associations between LTL and clinical parameters. Age, sex, BMI, smoking, alcohol, exercise, HDL-cholesterol, DM, CVD, HTN, and *FTO* genotype were included as variables. LTL was calculated after logarithmic transformation. A P value $<.05$ was considered statistically significant. All statistical analyses were performed using SPSS software, version 18.0 (SPSS Inc., Chicago, IL).

Table 1

Comparison of characteristics according to FTO rs9939609 genotype.

FTO rs9939609 genotype	TT	TA+AA	*P*
N	1636	497	
Age, y	58.4 ± 7.3	58.3 ± 7.5	.866
Male, n, %	822 (50.2)	267 (53.7)	.174
SBP, mm Hg	116.9 ± 24.1	116.4 ± 23.0	.694
FPG, mmol/L	5.4 ± 1.3	5.4 ± 1.1	.653
HbA1c, %*	5.6 (5.3–5.9)	5.6 (5.3–5.9)	.531
HbA1c, mmol/mol	38 (34–41)	38 (34–41)	.531
HOMA-IR*	1.8 (1.4–2.4)	1.8 (1.4–2.5)	.082
Total cholesterol, mmol/L	5.1 ± 0.9	5.1 ± 1.0	.918
HDL-cholesterol, mmol/L	1.3 ± 0.3	1.2 ± 0.3	.012
Triglyceride, mmol/L*	1.3 (1.0–1.8)	1.4 (1.0–2.0)	.094
Insulin, pmol/L*	52.1 (41.7–66.7)	54.2 (43.8–68.8)	.030
hsCRP, nmol/L*	5.7 (3.8–11.4)	6.7 (3.8–14.3)	.116
BMI, kg/m^2	24.6 ± 2.9	25.1 ± 3.0	.002
Waist circumference, cm	81.2 ± 8.5	82.5 ± 8.1	.002
Visceral fat area, cm^2	79.9 ± 37.0	85.1 ± 37.8	.010
Alcohol, current, n, %	791 (48.3)	232 (46.7)	.514
Smoking, current, n, %	202 (12.3)	69 (13.9)	.368
Regular exercise, n, %	569 (34.8)	167 (33.6)	.883
MetS, n, %	558 (34.2)	180 (36.4)	.374
CVD, n, %	102 (6.2)	43 (8.7)	.061
HTN, n, %	601 (36.7)	180 (36.2)	.834
DM, n, %	509 (31.8)	148 (30.3)	.547

Data are presented as number (%), mean ± SD, or median (interquartile range).

BMI = body mass index, CVD = cardiovascular disease, DM = diabetes mellitus, FPG = fasting plasma glucose, HbA1c = hemoglobin A1c, HDL = high-density lipoprotein, HOMA-IR = homeostasis model assessment of insulin resistance, hsCRP = high-sensitive C-reactive protein, HTN = hypertension, MetS = metabolic syndrome, SBP = systolic blood pressure.

* Statistical significance was estimated after logarithmic transformation.

3. Results

The clinical characteristics of the study subjects are listed in Table 1. Among the 2133 subjects, the proportions of the TT, TA, and AA genotypes were 76.7%, 21.5%, and 1.8%, respectively. The mean BMI was significantly higher in carriers of the A-risk allele than in subjects with the TT genotype (25.1 vs 24.6 kg/m^2, $P=.002$). Carriers of the risk allele had higher waist circumferences, visceral fat areas, insulin levels, and lower HDL-cholesterol levels than subjects without the risk allele. The other demographic and lifestyle-related variables were not significantly different between the genotypes.

Table 2 shows the characteristics of the participants stratified by the presence or absence of the *FTO* risk allele and obesity status. The subjects with obesity comprised 44.5% of the total subjects. The A-risk allele was identified in 21.9% of the subjects without obesity and 25.1% of the subjects with obesity. Among

Table 2

Characteristics of subjects according to obesity status and FTO rs9939609 genotype.

	BMI < 25 kg/m^2			BMI ≥25 kg/m^2		
	FTO rs9939609 genotype			*FTO* rs9939609 genotype		
	TT	TA + AA	*P*	TT	TA + AA	*P*
N	925	259		711	238	
Age, y	58.0 ± 7.2	58.5 ± 7.8	.377	58.8 ± 7.4	58.1 ± 7.2	.209
Male, n, %	430 (46.5)	144 (55.6)	.010	392 (55.1)	123 (51.7)	.355
SBP, mm Hg	115.6 ± 29.4	115.7 ± 29.3	.952	118.7 ± 14.3	117.2 ± 12.9	.152
FPG, mmol/L	5.3 ± 1.1	5.2 ± 0.9	.559	5.6 ± 1.5	5.6 ± 1.3	.678
HbA1c, %*	5.5 (5.3–5.8)	5.5 (5.3–5.8)	.635	5.6 (5.4–6.0)	5.6 (5.4–6.0)	.901
HbA1c, mmol/mol	37 (34–40)	37 (34–40)	.635	38 (36–42)	38 (36–42)	.901
HOMA-IR*	1.6 (1.2–2.0)	1.6 (1.2–2.0)	.509	2.1 (1.6–2.9)	2.2 (1.7–3.1)	.313
Total cholesterol, mmol/L	5.1 ± 0.9	5.1 ± 0.9	.309	5.1 ± 0.9	5.2 ± 1.0	.220
HDL-cholesterol, mmol/L	1.3 ± 0.3	1.3 ± 0.3	.038	1.2 ± 0.3	1.2 ± 0.3	.344
Triglyceride, mmol/L*	1.2 (0.9–1.7)	1.3 (0.9–1.8)	.666	1.5 (1.1–2.0)	1.5 (1.1–2.2)	.123
Insulin, pmol/L*	46.5 (38.9–58.3)	47.2 (39.6–57.6)	.338	60.4 (49.3–78.5)	61.1 (50.7–80.6)	.170
hsCRP, nmol/L*	4.8 (2.9–9.5)	5.7 (2.9–9.5)	.843	7.6 (4.8–14.3)	9.5 (4.8–17.1)	.028
BMI, kg/m^2	22.6 ± 1.6	22.9 ± 1.5	.003	27.3 ± 2.0	27.4 ± 2.2	.300
Waist circumference, cm	76.3 ± 6.4	77.9 ± 6.1	<.001	87.6 ± 6.5	87.5 ± 7.0	.799
Visceral fat area, cm^2	65.4 ± 30.9	71.6 ± 31.0	.007	99.0 ± 35.5	99.8 ± 39.1	.794
Smoking, current, n, %	102 (11.0)	45 (17.4)	.006	100 (14.1)	24 (10.1)	.115
Alcohol, current, n, %	414 (44.8)	116 (44.8)	.993	377 (53.0)	116 (48.7)	.252
Regular exercise, n, %	311 (33.7)	86 (33.2)	.988	258 (36.3)	81 (34.0)	.789
MetS, n, %	182 (19.7)	46 (17.8)	.492	376 (53.0)	134 (56.5)	.338
CVD, n, %	48 (5.2)	16 (6.2)	.534	54 (7.6)	27 (11.3)	.073
HTN, n, %	250 (27.0)	78 (30.1)	.326	351 (49.4)	102 (42.9)	.082
DM, n, %	254 (28.3)	74 (29.1)	.791	255 (36.2)	74 (31.6)	.202

Data are presented as number (%), mean ± SD, or median (interquartile range).

BMI = body mass index, CVD = cardiovascular disease, DM = diabetes mellitus, FPG = fasting plasma glucose, HbA1c = hemoglobin A1c, HDL = high-density lipoprotein, HOMA-IR = homeostasis model assessment of insulin resistance, hsCRP = high-sensitive C-reactive protein, HTN = hypertension, MetS = metabolic syndrome, SBP = systolic blood pressure.

* Statistical significance was estimated after logarithmic transformation.

nonobese subjects, those with the *FTO* risk allele had higher BMIs, waist circumferences, visceral fat areas, and lower HDL-cholesterol levels than noncarriers. Since they exhibited higher percentages of males and smokers than noncarriers, we also compared the carriers versus noncarriers after adjusting for age, sex, alcohol intake, and smoking status. After this adjustment, BMI, waist circumference, and visceral fat measurements were significantly higher in nonobese subjects carrying the *FTO* risk allele (Supplemental Table 1, http://links.lww.com/MD/B802). In the group with obesity, on the other hand, the *FTO* risk allele was not associated with BMI, waist circumference, or visceral fat areas. Only hsCRP level was higher in subjects with obesity and the *FTO* risk allele than in those without the risk allele.

Table 3 presents the LTL measurements according to *FTO* genotype. Subjects with the *FTO* risk allele had shorter LTLs than those without, even after adjustment for multiple confounding factors (0.96 vs 1.01, $P = .005$). These findings were only observed in the nonobese group and were observed whether or not adjustment for confounding factors was performed.

The Pearson correlations between LTL and clinical parameters are shown in Table 4. LTL was negatively correlated with age, HOMA-IR, insulin level, and waist circumference. In contrast,

Table 3

Comparison of leukocyte telomere length according to FTO rs9939609 genotype.

		FTO rs9939609		
		TT	TA + AA	*P**
Total	N	1636	497	
	Unadjusted	1.02 (1.00–1.04)	0.97 (0.94–1.00)	.002
	Adjusted†	1.01 (0.97–1.05)	0.96 (0.91–1.01)	.005
BMI < 25 kg/m^2	N	925	259	
	Unadjusted	1.03 (1.01–1.05)	0.95 (0.91–1.00)	.003
	Adjusted†	0.95 (0.90–1.01)	0.90 (0.84–0.96)	.030
BMI ≥25 kg/m^2	N	711	238	
	Unadjusted	1.01 (0.99–1.04)	0.98 (0.94–1.02)	.175
	Adjusted†	1.08 (1.02–1.14)	1.04 (0.97–1.11)	.142

Data are presented as geometric mean (95% CI).

† Adjusted for age, sex, BMI, smoking, alcohol, exercise, DM, CVD, and HTN.

BMI = body mass index, CVD = cardiovascular disease, DM = diabetes mellitus, HTN = hypertension.

* Statistical significance was estimated after logarithmic transformation.

Table 4

The correlations (*r*) between clinical parameters and natural logs of LTL values.

	Total		BMI < 25 kg/m^2		BMI ≥25 kg/m^2	
	r	*P*	*r*	*P*	*r*	*P*
Age, y	−0.055	.011	−0.084	.004	−0.018	.579
SBP, mm Hg	0.019	.378	0.016	.586	0.034	.300
FPG, mmol/L	0.002	.939	−0.007	.807	0.012	.709
HbA1c, %*	0.007	.731	−0.029	.311	0.048	.137
HOMA-IR*	−0.046	.035	−0.039	.181	−0.053	.103
Total cholesterol, mmol/L	0.009	.671	0.018	.527	−0.003	.929
HDL-cholesterol, mmol/L	0.055	.012	0.075	.010	0.022	.508
Triglyceride, mmol/L*	−0.042	.054	−0.028	.336	−0.057	.079
Insulin, pmol/L*	−0.058	.007	−0.047	.107	−0.073	.025
hsCRP, nmol/L*	−0.019	.387	0.001	.975	−0.043	.183
BMI, kg/m^2	−0.023	.284	−0.042	.151	−0.011	.740
Waist circumference, cm	−0.096	<.001	−0.130	<.001	−0.102	.002
Visceral fat area, cm^2	–0.019	.400	−0.050	.103	0.025	.464

BMI = body mass index, FPG = fasting plasma glucose, HbA1c = hemoglobin A1c, HDL = high-density lipoprotein, HOMA-IR = homeostasis model assessment of insulin resistance, hsCRP = high-sensitive C-reactive protein, SBP = systolic blood pressure.

* Statistical significance was estimated after logarithmic transformation.

LTL was positively correlated with the HDL-cholesterol level in total subjects. However, there was no association between BMI and LTL. In nonobese subjects, old age, high waist circumference, and low HDL-cholesterol level were associated with short telomere length. In subjects with obesity, on the other hand, high waist circumference and insulin level were associated with short LTL.

Stepwise multiple linear regression analyses were conducted to identify the independent variables associated with LTL (Table 5). After adjusting for several confounding factors including BMI, LTL was negatively correlated with age and the *FTO* risk allele and positively correlated with HDL-cholesterol level in total subjects. These relationships remained unchanged among nonobese subjects (Table 5). However, no factors were independently correlated with LTL in the subjects with obesity.

4. Discussion

In this study, we demonstrated differential associations between *FTO* genotype, metabolic risk factors, and LTL according to obesity status. Specifically, *FTO* was associated with higher BMI, waist circumference, and visceral fat measurements in the nonobese group. Moreover, the *FTO* risk allele was only significantly associated with short telomere length in nonobese subjects. To our knowledge, this study is the first to show that *FTO* has a greater metabolic impact in individuals without obesity than in those with obesity.

Table 5

Stepwise multiple linear regression analysis of the variables affecting LTL.

	Variable	β	*P*
Total	Age	−0.001	.009
	HDL-cholesterol	0.001	.028
	FTO	−0.022	.004
BMI < 25 kg/m^2	Age	−0.002	.007
	HDL-cholesterol	0.001	.038
	FTO	−0.027	.012
BMI ≥ 25 kg/m^2	None		

Age, sex, BMI, smoking, alcohol, exercise, HDL-cholesterol, DM, CVD, HTN, and *FTO* genotype were included as variables.

BMI = body mass index, CVD = cardiovascular disease, DM = diabetes mellitus, FTO = fat mass and obesity-associated, HDL = high-density lipoprotein, HTN = hypertension, LTL = leukocyte telomere length.

We found that *FTO* genotype was an independent variable affecting LTL. As mentioned above, *FTO* is of particular clinical interest because, beyond obesity, polymorphisms in this gene have been associated with several metabolic disorders and mortality.[5–7,9,10] However, little is known about how the biochemical function of *FTO* is related to obesity or metabolic abnormalities. Some studies have provided evidence that *FTO* risk allele carriers consume more food [26,27] and exhibit altered nutrient preference compared with noncarriers.[28] The *FTO* senses amino acid levels and couples it to mammalian target of rapamycin (mTOR) complex 1 signaling,[29] which functions as an energy sensor and controls protein synthesis. However, it is not yet known whether the mTOR pathway is relevant to the altered food intake and/or the increased cardiovascular risk discussed above.

On the other hand, it has been reported that the effect of the FTO obesity risk allele may be mediated through epigenetic changes. Most recently, it was reported that FTO rs9939609 risk allele was associated with increased obesity risk and short telomere length only in the high FTO methylation group in an Australian rural population.[30] Interestingly, this association was absent in the low FTO methylation group. FTO SNPs located within the first intron may also alter the expression of other downstream genes related to metabolic regulation. Almén et al[31] identified sites associated with the key genes Lysyl-tRNA synthetase (KARS), Telomeric repeat-binding factor 2-interacting protein 1 (TERF2IP), Dexamethasone-induced protein (DEXI), Musashi 1 (MSI1), Stonin 1 (STON1), and breast carcinoma amplified sequence 3 (BCAS3) that had a significant differential methylation level in the carriers of the FTO rs9939609 risk allele. Among these, certain genes, such as TERF2IP, [32] MSI1,[33] and STON1,[34,35] were known to be involved in telomere regulation. Thus, it can be possible that the FTO risk allele influences telomere shortening, through altering the expression of these genes.

We found that the metabolic effect of the *FTO* genotype differed according to obesity status. The association between *FTO* genotype and increased metabolic risk, including telomere shortening, was predominant only in nonobese subjects. In contrast, in subjects with obesity, the *FTO* polymorphism did not

exhibit any additive metabolic effect, with the exception that the elevated hsCRP level was observed in them. In particular, BMI was not significantly different between subjects with versus without the *FTO* risk allele in the group with obesity. The mechanism underlying this finding is unclear, because no study has yet compared the impact of the *FTO* risk allele according to adiposity. One potential explanation could be the smaller effect size and lower minor allele frequency of the *FTO* variant in Asian populations. FTO SNPs in Asian populations explain less of the variation in BMI (0.16–0.20%) than in Europeans (0.34%).[8] Thus, it could be inferred that the impact of the FTO risk allele might be stronger in the relationship between obesity and telomere shortening in Europeans than in Asians. Further investigations are necessary in diverse ethnic background to reveal the mechanism underlying the associations between *FTO* variants, metabolic obesity, and telomere shortening.

Contrary to our expectations, among the 4 groups, absolute LTL was shortest in nonobese subjects with the *FTO* risk allele (Table 3). That is, biological aging was most obvious in nonobese *FTO* risk allele carriers. This result is in line with the "obesity paradox," which refers to the finding that individuals with elevated BMI may have a lower mortality and better outcomes in several health parameters compared with subjects of normal weight.[36] The obesity paradox was clearly demonstrated by comparing future outcomes in subjects with MHO versus MONW.[18] Currently, we have been using the definition of metabolic syndrome, or HOMA-IR, or several other cardiovascular risk factors to define the metabolically abnormal group. However, there is a controversy in unifying definition of metabolic abnormality, which could fully account for future CVD or death. Since *FTO* is significantly associated with metabolic risk factors as well as short LTL, which reflects chromosomal instability leading to cell death and organ dysfunction, categorizing subjects by *FTO* may be an another option for defining MHO and MONW.

Our results also identify a problem with the current obesity cut-offs (BMI $\geq$ 25 kg/m^2 for Asians according to the Asia-Pacific guidelines [25] and $\geq$30 kg/m^{2}according to the World Health Organization).[37] Recently, however, it has been proposed that the obesity cut-off value should be revised to 27 kg/m^2 in Korea based on a nationwide mortality study.[38] The longer telomere length of the non-carriers with obesity supports this recommendation, because the mean BMI of the group with obesity was 27.3 kg/m^{2}in this study.

Several studies have shown significant associations between short telomere length and metabolic syndrome components.[39,40] Similarly, in our study, short LTL was correlated with high waist circumference and low HDL-cholesterol levels. In addition, short LTL was also correlated with high HOMA-IR and fasting insulin levels, which reflect insulin resistance. However, obesity (based on BMI) was not associated with telomere length in our study. In some previous studies, short LTL was found to be associated with BMI and percent body fat, [16,41,42] whereas others have not found any association.[43,44] It has been hypothesized that obesity accelerates telomere shortening due to high systemic oxidative stress and inflammation.[45] Rode et al[46] found an association between obesity and short telomere length, possibly mediated through elevated CRP levels in Europeans. Elevated CRP and other inflammatory markers were associated with short telomeres.[47] However, in our study, hsCRP levels were not correlated with telomere length both in nonobese and obese group. The inconsistencies observed in the associations between BMI and LTL imply that BMI may be an imperfect proxy measurement for adiposity. Moreover, the significant association between waist circumference and LTL observed in the present study indicates that central obesity may be a better marker for poor health outcomes.

In summary, biological aging may be accelerated by the *FTO* rs9939609 polymorphism in nonobese individuals. In our cohort of Koreans, we found that the metabolic impact of the *FTO* variant was more significant in nonobese subjects than in subjects with obesity. Our findings warrant further investigations into the biological mechanisms underlying this association. In addition, longitudinal follow-up studies assessing the prognostic implications of metabolically unhealthy people, as defined by the *FTO* genotype, could prove insightful.

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