Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 22 (2011) 252–258
Journal of Nutritional Biochemistry

A functional promoter polymorphism −607G>C of *WNT10B* is associated with abdominal fat in Korean female subjects☆

Il Chul Kim[a], Min Ho Cha[b], Dong Min Kim[a], Haeyong Lee[c], Jin Seok Moon[b], Sun Mi Choi[b], Kil Soo Kim[d], Yoosik Yoon[c,*]

[a]*Department of Biology, Chonnam National University, Gwangju 500-757, Republic of Korea*
[b]*Korea Institute of Oriental Medicine, Daejeon 305-811, Republic of Korea*
[c]*Department of Microbiology, Chung-Ang University College of Medicine, Seoul 156-756, Republic of Korea*
[d]*Kirin Oriental Hospital, Seoul 137-905, Republic of Korea*

Received 21 October 2009; received in revised form 22 January 2010; accepted 1 February 2010

Abstract

WNT10B has been implicated as a potential regulator of adipogenesis in cellular and animal models of obesity. In this study, we attempted to characterize the associations between common genetic polymorphisms of *WNT10B* and fat accumulation in a sample of 1029 Korean female subjects. Direct sequencing of genomic DNAs of 45 subjects identified six common single-nucleotide polymorphisms (SNPs) of *WNT10B*, which were in almost complete linkage disequilibrium. Among the six SNPs, −607G>C (rs833840) showed differential nuclear factor binding in an electrophoretic mobility shift assay and differential promoter activity in a reporter assay, implicating it as a functional regulatory SNP. When body compositions of the subjects determined using bio-impedance analysis were compared according to their −607G>C genotype, only body fat mass showed a significant association. Body masses of protein, mineral and water showed no association. For more accurate evaluation of the effects of −607G>C genotype on body fat, cross-sectional fat areas of the subjects measured by abdominal computed tomography were compared. Genotype of −607G>C was significantly associated with abdominal total fat and abdominal subcutaneous fat areas (P=.009 and P=.007 in recessive model, respectively). Of the 1029 subjects, 576 were treated with a 1 month very low calorie diet and changes of body weight and composition were compared with −607G>C genotype. No significant associations were evident. This study is the first report of the association of common genetic polymorphism of *WNT10B* with human fat accumulation.

Keywords: *WNT10B*; Polymorphism; SNP; Obesity; Fat; Association

1. Introduction

Wingless-type MMTV integration site (WNT) is a secreted glycoprotein involved in numerous events in human organogenesis, physiology and pathology [1,2]. The WNT signaling pathway involves binding to its receptor, Frizzled, and co-receptor, low-density lipoprotein receptor-related protein 5/6 (LRP5/6), which inhibits glycogen synthase kinase-3β and phosphorylation of β-catenin. The result is coactivation of transcription factors and expression of WNT target genes [3]. Decades of studies conducted in cellular and animal models have shown that the WNT pathway is involved in the suppression of adipogenesis and fat accumulation. *WNT10B*, a member of the WNT family, inhibits adipogenesis of 3T3-L1 preadipocytes through the inhibition of peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer binding protein α (C/EBPα) [4]. If WNT signaling is blocked, simultaneous fat cell differentiation occurs [5]. The role of *WNT10B* in fat regulation was elucidated by a study in transgenic mice, in which *WNT10B* was expressed under the fat tissue-specific FABP4 promoter [6]. The study reported that expression of *WNT10B* in a fat tissue-specific manner impairs fat accumulation throughout the body, with a decline of approximately 50% in total body fat and a reduction of approximately 60% in weight of epididymal and perirenal fat. These mice also resisted accumulation of fat tissue not only in high fat diet-induced obesity model but also in a genetic obesity model induced by ob/ob or agouti mutation [7]. On the other hand, the increased body fat observed in *WNT10B*−/− knockout mice has provided clear evidence of the suppressive effect of *WNT10B* on body fat accumulation [8].

Although *WNT10B* is a principal component of fat regulation in cellular and in animal models, its effect on humans has not been fully studied. There has been only one report on the effect of a rare mutation of *WNT10B* on the human obesity phenotype. In the study of Christodoulides et al. [9], five rare mutations of *WNT10B* (C256Y, P301S,

Abbreviations: BMI, body mass index; LD, Linkage disequilibrium; WHR, waist hip ratio; SNP, single nucleotide polymorphism; VLCD, very low calorie diet.

☆ This research was supported by grants from the Korea Institute of Oriental Medicine (KIOM, K09200) and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0065502).

* Corresponding author. Tel.: +82 2 820 5767; fax: +82 2 823 5423.
E-mail address: thanks@cau.ac.kr (Y. Yoon).

0955-2863/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2010.02.002

Table 1
Clinical and biochemical characteristics of study subjects

Variables	*n*	Mean±S.D.
Age (year)	1029	28.27±8.14
Body weight (kg)	1029	66.49±11.53
BMI (kg/m^2)	1029	25.72±4.07
WHR	1029	0.880±0.066
Body fat mass (kg)	1029	23.49±7.79
Body protein mass (kg)	1029	9.68±1.78
Body mineral mass (kg)	1029	2.73±0.39
Body water mass (kg)	1029	30.87±4.05
Serum total cholesterol (mg/dl)	1011	175.47±64.39
Serum triglyceride (mg/dl)	1017	81.90±49.61
Fasting serum glucose (mg/dl)	1014	108.22±33.85
Systolic blood pressure (mm Hg)	1029	115.85±34.75
Diastolic blood pressure (mm Hg)	1029	72.16±10.76
VLCD-induced body weight change (kg)	576	−5.93±2.66
VLCD-induced fat mass change (kg)	576	−3.96±2.35
VLCD-induced protein mass change (kg)	576	−0.51±0.69
VLCD-induced mineral mass change (kg)	576	−0.078±0.136
VLCD-induced water mass change (kg)	576	−1.34±1.50

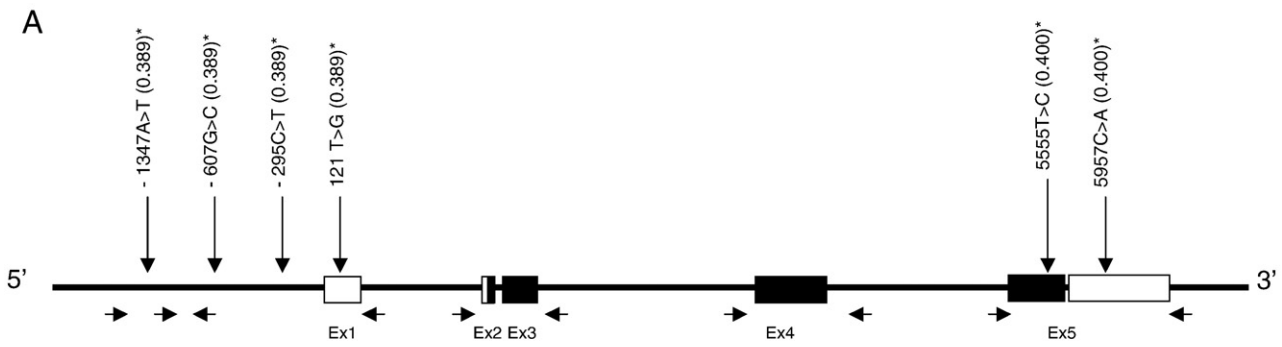
H77Y, I285T, and H353H) were detected in Caucasian subjects, with C256Y cosegregating with obesity in family studies. Its low allele frequency in severely obese subjects (<0.5%) and absence in 100 normal subjects shows that it is a very rare mutation affecting human obesity [9].

In this study, we investigated the associations between common genetic polymorphisms of *WNT10B* and fat accumulation in human subjects. Six common single nucleotide polymorphisms (SNPs) were identified in *WNT10B*, and the association of the SNP with body fat was analyzed in 1029 Korean females. The results are the first demonstration of an association of a common genetic polymorphism of *WNT10B* with human fat accumulation.

2. Research design and methods

2.1. Subjects

Subjects were recruited from a women's health and obesity clinic at Kirin Oriental Medical Hospital (Seoul, Korea). Subjects with diabetes, hypertension and liver/renal function disorders were excluded to find the genetic factors for fat accumulation and to exclude other factors such as inflammation, oxidative stress and hormonal abnormality which influence metabolic syndrome [10]. A total of 1029 female subjects were included. The subjects of this study partially overlapped with our previous study [11]. Female subjects aged >50 years with a high possibility of menopause comprised only 2.2% of the total subjects, and their exclusion did not significantly affect the results. The clinical and biochemical characteristics of the subjects are listed in Table 1. Body composition parameters including fat, protein, minerals and water were measured by bio-impedance using an Inbody 2.0 device (Biospace, Seoul, Korea). Blood sample obtained from each subject after an overnight fast was centrifuged for 10 min at 2000 rpm to separate serum. Serum biochemical profiles were measured with a model SP-4410 auto-biochemical analyzer (ARKRAY, Kyoto, Japan). Areas of abdominal subcutaneous and visceral fat were measured by Hispeed CT/e computed tomography (CT) unit (General Electric Medical Systems, Waukesha, WI, USA) running on 120 kV_p, 200 mA, 2.0 s scan time and 10 mm slice thickness. Each subject was scanned by CT on the abdominal position at the level of the umbilicus. The cross-sectional area of adipose tissue was measured in mm^2 assuming a density of −40 to −140 Hounsfield units for adipose tissue as described [12]. Total abdominal fat area was calculated as the sum of visceral fat area and subcutaneous fat area. Of the subjects, 576 finished a 1-month weight control program



B

|D'|

r^2	-1347 A>T	-607 G>C	-295 C>T	121 T>G	5555 T>C	5957 C>A
-1347 A>T		1	1	1	1	1
-607 G>C	1		1	1	1	1
-295 C>T	1	1		1	1	1
121 T>G	1	1	1		1	1
5555 T>C	0.995	0.995	0.995	0.995		1
5957 C>A	0.995	0.995	0.995	0.995	1	

C

Hap.	-1347 A>T	-607 G>C	-295 C>T	121 T>G	5555 T>C	5957 C>A	Freq.
ht 1	A	G	C	T	T	C	0.600
ht 2	T	C	T	G	C	A	0.389
ht 3	A	G	C	T	C	A	0.011

Fig. 1. SNPs and haplotypes of *WNT10B* in Korean subjects. (A) Gene map of *WNT10B* and the location of six SNPs identified by genomic sequencing of 45 Korean subjects. Coding exons are marked by black blocks and 5′ and 3′ UTRs are marked by white blocks. The minor allele frequencies are shown in parentheses. PCR primers used for genomic amplification and sequencing are shown below the gene map. (B) LD coefficients among the six SNPs of *WNT10B*. (C) Haplotypes constructed from the six SNPs of *WNT10B* and their frequencies.

Table 2
Characteristics of SNPs identified by genomic sequencing of the promoter, exon and exon-intron boundary regions of *WNT10B* in 45 Korean subjects

SNP	Position	Location relative to transcription start site	Nucleotide (amino acid) change	Location relative to p terminus of chromosome 12	Minor allele frequency	HWE *P*
rs833839	Promoter	−1347	A>T	47,653,157	0.389	.257
rs833840	Promoter	−607	G>C	47,652,417	0.389	.257
rs833841	Promoter	−295	C>T	47,652,105	0.389	.257
rs833842	5′UTR	+121	T>G	47,651,690	0.389	.257
rs3741627	Exon 5	+5555	T>C (His>His)	47,645,854	0.400	.172
rs1051886	3′UTR	+5957	C>A	47,646,256	0.400	.172

composed of 700 Kcal/day very low calorie diet (VLCD). Each subject was provided with essential food to maintain vigor during the VLCD and with nutritionist-aided counseling using a diet diary. Body weight and body compositions measurements were recorded before and after the weight control program, and the changes were calculated. Blood samples and medical records were obtained with informed consent. The study protocol was approved by the Institutional Review Board of Korea Institute of Oriental Medicine.

2.2. Identification of SNP

Genomic DNA was extracted from whole blood using an Accuprep Genomic DNA Extraction Kit (Bioneer, Daejon, Korea). All exons, exon-intron boundaries and the promoter region (1.5 kb) of the *WNT10B* gene were sequenced using an ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Genomic sequencing was conducted for 45 Korean genomic DNA samples (90 haploid genomes), which was sufficient to discover common genetic polymorphisms (frequency>0.05). Primer sets used in the amplification and sequencing analyses were designed on the basis of reference genome sequence for *WNT10B* : NT_086693. Information concerning the primers for the amplification and sequencing of the *WNT10B* gene is shown in Supplemental data 1. Hardy-Weinberg equilibrium tests were employed to determine whether individual SNPs were in equilibrium at each locus, and linkage disequilibrium (LD) coefficients, |D'| and r^2, were evaluated in order to measure LD between all pairs of loci [13]. Haplotypes and their frequencies were inferred using the HapAnalyzer program (http://hap.ngri.go.kr).

2.3. Reporter assay of WNT10B promoter

The promoter region of *WNT10B* was amplified by polymerase chain reaction (PCR) using a forward primer, 5′-CGGGGTACCATGGCTTGCCAAAACAATCTC-3′, and a reverse primer, 5′- TGCTCTAGATTACTCGATGGCACTAAACTC-3′. The cytomegalovirus promoter of pcDNA3 containing the luciferase(Luc) gene (Invitrogen Life Technologies, Carlsbad, CA, USA) was replaced with the amplified *WNT10B* promoters, which resulted in *WNT10B*-promoter-Luc plasmid. For luciferase reporter assay, 0.4 μg of *WNT10B*-promoter-Luc plasmid and 0.2 μg of Renilla luciferase plasmid were cotransfected into NIH3T3 cells cultured in Dulbecco's modified Eagle's medium (DMEM; Life Technologies Inc., Grand Island, NY, USA). Luciferase assays were performed 48 h after transfection using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) and a Lumat LB9501 luminometer (Berthold, Bad Wildbad, Germany). The luminescence value of *WNT10B*-promoter-Luc was normalized by the luminescence value of Renilla luciferase. Transfections were performed in triplicate and repeated three times.

2.4. Electrophoretic mobility shift assay (EMSA) of promoter SNPs

Nuclear proteins were extracted from 3T3-L1 preadipocytes possessing all the factors for the expression of *WNT10B* [4,14]. 3T3-L1 cells were cultured in DMEM and were dissolved in buffer A (25 mM Tris-HCl (pH 7.5), 50 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA, 5 mM dithiothreitol) and homogenized in a tightly-sealed homogenizer. The homogenate was pelleted by centrifugation at 3,300×g for 10 min and washed twice with buffer A. High salt extraction of nuclear proteins was performed by incubating nuclei with buffer B (25 mM Tris-HCl (pH 7.5), 420 mM NaCl, 1.5 mM $MgCl_2$, 1 mM dithiothreitol, 0.5 mM EDTA, 25% sucrose) for 30 min on ice. After centrifugation at 20,000×g for 30 min, the supernatant was used as the nuclear extract, and its protein concentration was determined using a bicinchoninic acid-based protein assay kit (Pierce, Rockford, IL, USA). Primer sets containing each allele of SNP (Supplemental data 2) were biotin-labeled at the 5′ position. Each forward and reverse primer set was annealed by heating at 95°C for 5 min and cooling slowly to room temperature. Ten nanograms of biotin-labeled primer, 10 μg of nuclear extract protein, and 1 μg of poly d(I-C) were incubated at 15°C for 30 min in a final volume of 10 μl. For competition experiments, 660 ng of non-labeled primers were also added. The reaction mixtures were separated by 6.0% non-denaturing polyacrylamide gel electrophoresis at 120 V in Tris-borate-EDTA (TBE) buffer and electrotransferred to a nylon membrane at 300 mA for 30 min. The locations of protein/DNA complexes were visualized by incubation using horseradish peroxidase-conjugatedstreptavidin and an enhanced chemiluminescence kit (Pierce).

2.5. Genotyping of selected SNP

Genotyping of −607G>C (rs833840) polymorphism was conducted using the TaqMan method [15]. The sequences of forward and reverse amplification primers and two genotyping probes were:

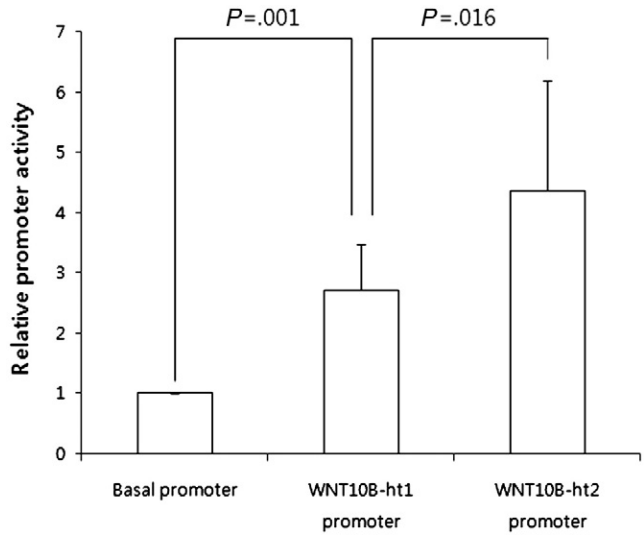


Fig. 2. Promoter reporter assay of two common haplotypes of *WNT10B*. Promoter regions of *WNT10B* were amplified by PCR using genomic DNAs of ht1 homozygote and ht2 homozygote and were inserted into pcDNA-Luc plasmid. Promoter activities were measured by luciferase activities after transfection, and expressed by relative values compared with the activity of basal promoter.

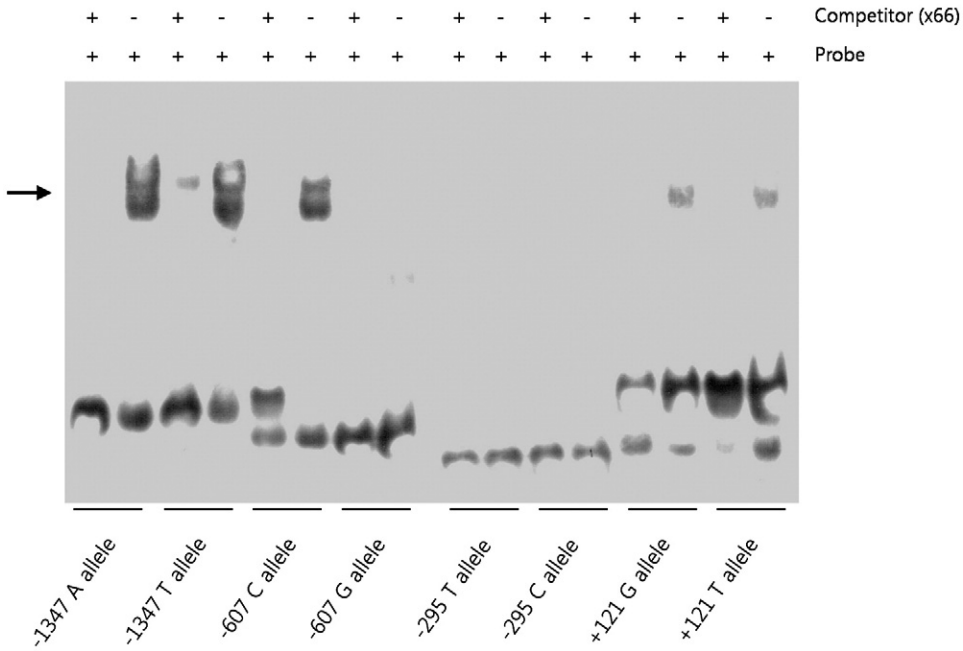


Fig. 3. Effect of promoter polymorphisms of *WNT10B* on nuclear factor binding. EMSA was conducted with nuclear extract prepared from 3T3-L1 preadipocytes. Biotin-labeled double-stranded DNA primers and excess of non-labeled competitor primers containing each alleles of 4 SNPs were used. The arrow indicates the band of specific complex that was formed by the binding of the biotin-labeled primers and nuclear protein factors.

5′-TGGCCCTAGCAGAGGTTCAAGTA -3′ (forward primer)
5′-TCCCGAGAAGGGTAGGATAC-3′ (reverse primer)
5′-FAM-TCAGCCTGCCCCGATGCTT- 3′ (genotyping probe for G allele)
5′-VIC-TCAGCCTGCGCCGATGCTT- 3′ (genotyping probe for C allele)

PCR was typically run in a TaqMan Universal Master mix (Applied Biosystems) at a primer concentration of 900 nM and a probe concentration of 200 nM. The reactions were conducted in a 5 μl total reaction volume containing 20 ng of genomic DNA. The TaqMan assay plate was then placed in a PE 9700 thermal cycler (Applied Biosystems) and heated for 10 min at 95°C followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Fluorescence data files from each of the plates were analyzed using SDS 2.1 software (Applied Biosystems).

2.6. Statistical analyses

Association analyses were conducted using the General Linear Model procedures. Age-adjusted univariate analyses of variance were performed to delineate the effects of the *WNT10B* genotype on obesity phenotypes, fat accumulation and biochemical profiles. A *P* value of <.05 was considered to be statistically significant. It is usual that some procedures including Bonferroni correction and false discovery rate are used to account for multiple comparisons carried out with multiple SNPs, and they were properly used in our previous study on the genetic makers of obesity-related phenotypes [16–18]. In this study, however, all six SNPs were in complete linkage disequilibrium, which means complete dependence among them, as shown in Fig. 1B. One functional SNP, −607G>C, was selected from 6 SNPs for genotyping and used as an independent variable in all statistical analyses, showing that the correction for multiple comparison was not necessary. Statistical analyses were conducted using the SPSS software package 17.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. SNPs and haplotypes of WNT10B in Korean subjects

Through the sequencing of the promoter, exons and exon-intron boundary regions of *WNT10B* gene in 45 Korean subjects, six SNPs were identified. The raw data of sequencing profiles is shown in Supplemental data 3, and the genotypes of 45 subjects in these 6 SNP sites are listed in Supplemental data 4. The

Table 3
Association analyses of -607 G>C with anthropometric parameters

Phenotype	−607G>C			*P*-value[a]		
	GG	GC	CC	Codominant	Dominant	Recessive
Body weight (kg)	264[b] (66.60±11.58)	509 (67.00±12.24)	256 (65.35±9.87)	.195[c]	.849	.079
BMI (kg/m^2)	264 (25.69±3.82)	509 (25.99±4.40)	256 (25.23±3.56)	.079	.905	**.038**
WHR	264 (0.879±0.062)	509 (0.885±0.071)	256 (0.870±0.057)	**.021**	.824	**.011**

[a] *P* values of three alternative models (codominant, dominant and recessive) of age-adjusted univariate analyses of variance are shown.
[b] Number of subjects (mean±S.D.).
[c] *P* values <.05 are marked by bold characters.

Table 4
Association analyses of −607G>C with body composition

Phenotype	−607G>C			P[a]		
	GG	GC	CC	Codominant	Dominant	Recessive
Body fat mass (kg)	264[b] (23.26±7.27)	509 (23.86±8.12)	256 (22.41±6.40)	**.046**[c]	.843	**.024**
Body protein mass (kg)	264 (9.72±1.82)	509 (9.62±1.79)	256 (9.73±7.71)	.667	.627	.584
Body mineral mass (kg)	264 (2.74±0.40)	509 (2.74±0.40)	256 (2.71±0.36)	.659	.872	.368
Body water mass (kg)	264 (30.96±4.28)	509 (30.82±3.93)	256 (30.66±3.59)	.720	.485	.533

[a] *P* values of three alternative models (codominant, dominant and recessive) of age-adjusted univariate analyses of variance are shown.
[b] Number of subjects (mean±S.D.).
[c] *P* values <.05 are marked by bold characters.

location of the six SNPs is shown in Fig. 1A and their characteristics are listed in Table 2. These SNPs were in the promoter (n=3), in the 5′ untranslated region (n=1), 3′ untranslated region (n=1) and in exon 5 with no amino acid change (n=1). All were in Hardy Weinberg equilibrium (P>.05). The LD coefficients between the six SNPs were |D'|=1 and r^2≥0.995, which suggested that all six SNPs were in complete LD in the Korean population (Fig. 1B). Haplotypes were constructed as shown in Fig. 1C. Two common haplotypes, ht1 and ht2, were found. Ht3 with allele frequency of 0.011 was not analyzed further because we are interested in common genetic polymorphisms (frequency≥0.05). The results showed that ht1 was tagged by major alleles of the six SNPs and ht2 was tagged by minor alleles. Because of complete LD, one SNP could explain the effects of the other five SNPs and two common haplotypes.

3.2. Effects of WNT10B polymorphisms on promoter activity and nuclear factor binding

The effects of *WNT10B* haplotypes on gene expression levels were measured by a promoter reporter assay (Fig. 2). The ht2 haplotype displayed significantly higher promoter activity than the ht1 haplotype, suggesting that subjects with ht2 haplotype had increased *WNT10B* expression level compared with those with ht1 haplotype. Four SNPs, -1347A>T, −607G>C, -295C>T and +121T>G, were located in the 5′ flanking region of the gene that might be involved in the regulation of transcription. EMSA assays were conducted to elucidate the effects of these four SNPs on nuclear factor binding. Only −607G>C had differential binding activity (Fig. 3). The −607C allele showed nuclear factor binding but the −607G allele was not bound. Nuclear factors were bound at the location of −1347A>T and +121T>G, but their binding activities did not differ between two alleles of each SNP. No bound nuclear factor binding was evident at the location of -295C>T. The data implicated −607G>C as a functional regulatory SNP affecting promoter activity through differential effects on nuclear factor binding. Among the six SNPs in complete LD, −607G>C (rs833840) was selected for genotyping of all subjects and was analyzed for phenotype association.

3.3. Associations of −607G>C with body fat accumulation

Table 3 shows the comparison of obesity phenotypes by −607G>C genotypes. Obesity indices such as body mass index (BMI) and waist-to-hip ratio (WHR) showed an association with −607G>C genotype (P=.038 and P=.011 in recessive model, respectively). CC homozygotes showed reduced BMI and WHR compared with GG homozygotes and heterozygotes. When body compositions were compared with −607G>C genotypes, only body fat mass showed an association (P=.024 in recessive model), and CC homozygotes displayed less body fat compared with GG and GC genotypes (Table 4). Other body components including protein, minerals and water showed no significant differences according to genotype. These results are consistent with the suggestion that the effects of −607G>C genotype on obesity indices summarized in Table 3 are mediated by effects on body fat rather than non-fat components. For a more accurate evaluation of the effects of −607G>C genotype on body fat, cross sectional abdominal fat areas of all subjects measured by CT were compared. Total abdominal fat area was significantly associated with the −607G>C genotypes (P=.009 in recessive model) (Table 5). Among the two components of abdominal fat, subcutaneous fat area was significantly associated with −607G>C genotypes (P=.007 in recessive model). Total abdominal fat and subcutaneous fat areas were about 11% less on average in subjects with CC genotype compared with GG or GC genotypes. Mean fat areas of GC heterozygotes were similar to those of GG homozygotes, suggesting a recessive nature for the C alleles; this was also shown by the lowest P value of the recessive model. When serum biochemical profiles were compared, serum glucose level showed significant association with −607G>C genotypes (P=.013 in recessive model) (Table 6). Among the subjects who finished a 1-month weight control program composed of VLCD and nutritional counseling, the changes of body weight and body compositions were not significantly associated with −607G>C genotypes (Table 7).

4. Discussion

WNT10B has been implicated as a potential regulator of adipocyte differentiation [4–8]. Even though many cellular and

Table 5
Association analyses of -607 G>C with CT-measured fat areas

Phenotype	−607G>C			P[a]		
	GG	GC	CC	Codominant	Dominant	Recessive
Total abdominal fat area (mm^2)[b]	263[c] (28559.1±729.6)	504 (29255.5±575.0)	253 (26576.6±646.6)	**.026**[d]	.796	**.009**
Visceral fat area (mm^2)	263 (5930.2±222.7)	504 (6245.81±80.0)	253 (5641.8±194.1)	.291	.740	.182
Subcutaneous fat area (mm^2)	263 (22540.0±584.4)	504 (23009.7±472.0)	253 (20934.7±530.3)	**.020**	.775	**.007**

[a] *P* values of three alternative models (codominant, dominant and recessive) of age-adjusted univariate analyses of variance are shown.
[b] Total abdominal fat area is the sum of visceral fat area and subcutaneous fat area.
[c] No. of subjects (mean±S.D.).
[d] *P* values <.05 are marked by bold characters.

Table 6
Association analyses of −607G>C with serum biochemical profiles

Phenotype	−607G>C			*P* value[a]		
	GG	GC	CC	Codominant	Dominant	Recessive
Glucose (mg/dl)	257[b] (107.20±31.36)	500 (110.93±36.63)	252 (103.18±29.39)	**.018**[c]	.658	**.013**
HDL cholesterol (mg/dl)	226 (57.99±27.01)	436 (58.31±25.02)	228 (56.35±21.97)	.659	.846	.365
Total cholesterol (mg/dl)	255 (177.79±71.65)	498 (174.89±62.24)	253 (174.54±59.14)	.794	.497	.855
Triglyceride (mg/dl)	258 (80.61±49.67)	501 (81.19±50.04)	253 (84.57±49.04)	.461	.643	.213

[a] *P* values of three alternative models (codominant, dominant and recessive) of age-adjusted univariate analyses of variance are shown.
[b] Number of subjects (mean±S.D.).
[c] *P* values <.05 are marked by bold characters.

animal studies have supported an anti-adipogenic effect of *WNT10B* in humans, until now no study has addressed the common genetic polymorphisms of *WNT10B* in relation to fat accumulation. Several cell types including adipocytes, myocytes, chondrocytes and osteoblasts differentiate from a common precursor, the pluripotent mesenchymal stem cell [19]. The WNT signaling pathway alters the fate of mesenchymal stem cells which have the potential to differentiate into several cell types, and the expression of *WNT10B* suppresses the differentiation into adipocytes [20]. Fat accumulation is the result of the adipocyte differentiation, and the expression levels of *WNT10B* can modulate fat accumulation by the modulation of the degree of mesenchymal stem cell differentiation into adipocyte lineage. In this study, we attempted to characterize the possible effects of *WNT10B* polymorphisms on human body fat accumulation, and found, for the first time, that *WNT10B* polymorphism was associated with body fat mass and abdominal fat tissue areas (Tables 4 and 5). It should be noted that *WNT10B* is expressed in preadipocytes but its expression declines during adipocyte differentiation. *WNT10B* is not expressed in fully differentiated adipocytes suggesting that *WNT10B* is involved in the formation of adipocytes but has no roles in fully differentiated adipocytes [4]. Until now, no reports were found on the involvement of *WNT10B* in fat mobilization of fully differentiated adipocytes, and it looks like that VLCD-induced fat mobilization has no relation with differentiation process in which *WNT10B* is involved. Our data show that *WNT10B* polymorphism was not associated with VLCD-induced body fat changes (Table 7).

It has been reported that the expression of *WNT10B* suppresses adipogenic differentiation and activates osteogenic differentiation by induction of the osteoblastogenic transcription factors Runx2, Dlx5 and osterix, and suppression of the adipogenic transcription factors C/EBPα and PPARγ [21]. *WNT10B* over-expressing transgenic mice have elevated bone mass and are protected from the bone loss characteristic of estrogen deficiency, while, on the other hand, *WNT10B*−/− mice have decreased bone mass, confirming that *WNT10B* is an endogenous activator of bone formation [22]. Some genetic association studies of *WNT10B* were conducted in relation to bone metabolism with controversial results. In Afro-Caribbean men, *WNT10B* polymorphisms are associated with bone mineral density [23], while, contrarily, in Caucasian postmenopausal women, a common *WNT10B* polymorphism was not found to be associated with bone mineral density and fractures [24]. In the present study, in which most subjects were premenopausal women, *WNT10B* polymorphism showed no associations with body mineral mass, which is related with bone metabolism (Table 4).

Among six SNPs of *WNT10B* identified in Korean subjects, −607G>C (rs833840) exhibited differential nuclear factor binding behavior in EMSA and differential promoter activity in a reporter assay; both results are evidence of a functional regulatory SNP (Figs. 2 and 3). It is likely that -607C>G SNP, located in the promoter region, affects the level of *WNT10B* gene expression by differential binding with transcription factors. Possible transcription factors that can bind at the −607G>C site of the *WNT10B* promoter were searched using MATCH public version 1.0 (http://www.gene-regulation.com/pub/programs.html#match) [25]. The result of the database search showed that the possible binding sites of the transcription factors including Sp1 (AGCCTGCCCC), GATA-1 (CCCGATGCTT), GKLF (CCTGCCCCGATGCT), MZF1 (CTGCCCCGATGCT), AP2 (GCCCCGATGCTT) and so on were located at −607 position of the promoter (The underlined C is the position of the −607G>C SNP, and the C allele harbored the transcription factor binding site, but the G allele did not contain a binding site). The differential transcription factor binding provides a plausible mechanism of −607G>C SNP, which modifies the expression of *WNT10B* and regulates body fat content. More studies are needed to elucidate the identities and molecular mechanisms of the transcription factors which bind at −607 position of the promoter. It also remains to be elucidated why *WNT10B* polymorphism was significantly associated with abdominal subcutaneous fat but was not associated with visceral fat (Table 5). Although the findings of this study should be interpreted within the context of its limitations, and may be not appropriate for extrapolation to male subjects, the results provide important clues regarding the role of *WNT10B* in human fat metabolism.

Table 7
Association analyses of −607G>C with VLCD-induced changes in body weight and body composition

Phenotype	−607G>C			*P*[a]		
	GG	GC	CC	Codominant	Dominant	Recessive
Body weight (kg)	148[b] (−5.60±2.55)	279 (−6.09±2.84)	149 (−5.97±2.40)	.172	.071	.872
Body fat mass (kg)	148 (−3.64±2.55)	279 (−4.14±2.66)	149 (−3.94±1.74)	.124	.060	.934
Body protein mass (kg)	148 (−0.52±0.72)	279 (−0.51±0.74)	149 (−0.50±0.55)	.983	.885	.874
Body mineral mass (kg)	148 (−0.079±0.134)	279 (−0.073±0.148)	149 (−0.087±0.113)	.631	.993	.367
Body water mass (kg)	148 (−1.35±1.49)	279 (−1.29±1.62)	149 (−1.41±1.23)	.816	.939	.571

[a] *P* values of three alternative models (codominant, dominant and recessive) of age-adjusted univariate analyses of variance are shown.
[b] Number of subjects (mean±S.D.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2010.02.002.

References

[1] Willert K, Brown JD, Danenberg E, Duncan AW, Weissman IL, Reya T, et al. Wnt proteins are lipid-modified and can act as stem cell growth factors. Nature 2003;423:448–52.
[2] Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004;20:781–810.
[3] Hagen T, Sethi JK, Foxwell N, Vidal-Puig A. Signalling activity of beta-catenin targeted to different subcellular compartments. Biochem J 2004;379(Pt 2):471–7.
[4] Bennett CN, Ross SE, Longo KA, Bajnok L, Hemati N, Johnson KW, et al. Regulation of Wnt signaling during adipogenesis. J Biol Chem 2002;277:30998–1004.
[5] Ross SE, Hemati N, Longo KA, Bennett CN, as PC, Erickson RL, et al. Inhibition of adipogenesis by Wnt signaling. Science 2000;289:950–3.
[6] Longo KA, Wright WS, Kang S, Gerin I, Chiang SH, as PC, et al. Wnt10b inhibits development of white and brown adipose tissues. J Biol Chem 2004;279:35503–9.
[7] Wright WS, Longo KA, Dolinsky VW, Gerin I, Kang S, Bennett CN, et al. Wnt10b inhibits obesity in ob/ob and agouti mice. Diabetes 2007;56:295–303.
[8] Vertino AM, Taylor-Jones JM, Longo KA, Bearden ED, Lane TF, McGehee Jr RE, et al. Wnt10b deficiency promotes coexpression of myogenic and adipogenic programs in myoblasts. Mol Biol Cell 2005;16:2039–48.
[9] Christodoulides C, Scarda A, Granzotto M, Milan G, Dalla Nora E, Keogh J, et al. WNT10B mutations in human obesity. Diabetologia 2006;49:678–84.
[10] Ferder L, Inserra F, Martinez-Maldonado M. Inflammation and the metabolic syndrome: role of angiotensin II and oxidative stress. Curr Hypertens Rep 2006;8:191–8.
[11] Kim KS, Cho DY, Kim YJ, Choi SM, Kim JY, Shin SU, et al. The finding of new genetic polymorphism of UCP-1 A-1766G and its effects on body fat accumulation. Biochim Biophys Acta 2005;1741:149–55.
[12] Tokunaga K, Matsuzawa Y, Ishikawa K, Tarui S. A novel technique for the determination of body fat by computed tomography. Int J Obes 1983;7:437–45.
[13] Hedrick PW. Gametic disequilibrium measures: proceed with caution. Genetics 1987;117:331–41.
[14] Fox KE, Colton LA, Erickson PF, Friedman JE, Cha HC, Keller P, et al. Regulation of cyclin D1 and Wnt10b gene expression by cAMP-responsive element-binding protein during early adipogenesis involves differential promoter methylation. J Biol Chem 2008;283:35096–105.
[15] Livak KJ. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal 1999;14:143–9.
[16] Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995;310:170.
[17] Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behav Brain Res 2001;125:279–84.
[18] Cha MH, Kim IC, Kim KS, Kang BK, Choi SM, Yoon Y. Association of UCP2 and UCP3 gene polymorphisms with serum high-density lipoprotein cholesterol among Korean women. Metabolism 2007;56:806–13.
[19] Li HX, Luo X, Liu RX, Yang YJ, Yang GS. Roles of Wnt/beta-catenin signaling in adipogenic differentiation potential of adipose-derived mesenchymal stem cells. Mol Cell Endocrinol 2008;291:116–24.
[20] Takada I, Kouzmenko AP, Kato S. Molecular switching of osteoblastogenesis versus adipogenesis: implications for targeted therapies. Expert Opin Ther Targets 2009;13:593–603.
[21] Kang S, Bennett CN, Gerin I, Rapp LA, Hankenson KD, Macdougald OA. Wnt signaling stimulates osteoblastogenesis of mesenchymal precursors by suppressing CCAAT/enhancer-binding protein alpha and peroxisome proliferator-activated receptor gamma. J Biol Chem 2007;282:14515–24.
[22] Bennett CN, Longo KA, Wright WS, Suva LJ, Lane TF, Hankenson KD, et al. Regulation of osteoblastogenesis and bone mass by Wnt10b. Proc Natl Acad Sci U S A 2005;102:3324–9.
[23] Zmuda JM, Yerges LM, Kammerer CM, Cauley JA, Wang X, Nestlerode CS, et al. Association analysis of WNT10B with bone mass and structure among individuals of African ancestry. J Bone Miner Res 2009;24:437–47.
[24] Perez-Castrillon JL, Olmos JM, Nan DN, Castillo J, Arozamena J, Montero A, et al. Polymorphisms of the WNT10B gene, bone mineral density, and fractures in postmenopausal women. Calcif Tissue Int 2009;85:113–8.
[25] Kel AE, Gossling E, Reuter I, Cheremushkin E, Kel-Margoulis OV, Wingender E. MATCH. A tool for searching transcription factor binding sites in DNA sequences. Nucleic Acids Res 2003;31:3576–9.