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Research Article

Validation of G-protein beta-3 subunit gene C825T polymorphism as predictor of obesogenic epidemics in overweight/obese Korean children*

Lee, Yunkyoung[1] · Park, Seong-min[2,3] · Lee, Myoungsook[3†]

[1]Department of Food Science and Nutrition, Jeju National University, Jeju 63243, Korea
[2]Imported Food Analysis Team, Ministry of Food Drug Safety, Gyeongin Regional, Food & Drug Administration, Gyeonggi 13809, Korea
[3]Department of Food and Nutrition and Research Institute of Obesity Sciences, Sungshin Women's university, Seoul 01133, Korea

ABSTRACT

Purpose: We investigated the potential interaction between the G-protein beta-3 subunit gene (GNB3) C825T polymorphism, a risk factor for chronic disease in various ethnicities, and obesogenic environments in overweight/obese Korean children. **Methods:** The present study was conducted as a cross-sectional study using measures of anthropometry, blood pressure (BP), and fasting blood samples as well as 3-day food records. Subjects were recruited from seven elementary schools in an urban district in Seoul, South Korea, between 2007 and 2008. A total of 1,260 children aged 8-9 years were recruited in the study, including 633 boys (50.3%) and 627 girls (49.7%). **Results:** The allele frequencies of the GNB3 polymorphism were C allele = 49.7% and T allele = 50.3% in subjects. In general, boys with T allele had higher BMI, systolic BP (SBP), and triglycerides, although their energy intake was not significantly different from boys with C allele. In contrast to boys, girls with T allele had lower BMI but higher SBP and energy intake than those with C allele. The girls with T allele had a significantly lower BMI and waist circumference in both the normal weight group and obese group (OB). T allele carriers in both genders had significantly higher TC than C allele carriers in the OB group. At last, girls with T allele in OB appeared to have significantly lower HOMA-IR than those with C allele. **Conclusion:** Unlike higher risk for negative health outcomes by the GNB3 polymorphism in various ethnicities, GNB3 polymorphism did not influence obesogenic environments in overweight/obese Korean children.

KEY WORDS: children obesity, gender, GNB3 C825T polymorphism, obesogenic

INTRODUCTION

Childhood obesity has been continuously increasing worldwide. According to the Korean National Health and Nutrition Examination Survey conducted in 2010, the prevalence of obesity in Korean children dramatically increased from approximately 5% to 20% during the last two decades.[1] Childhood obesity is strongly associated with several health conditions, including cardiovascular diseases, cancers, diabetes, high BP, and dyslipidemia. Therefore, the prevention of early onset of obesity is a critical factor to control serious consequences in adults.[2] Various factors including genetic, behavioral, environmental, physiological, social factors, contribute the etiology of obesity.[3] In fact, it was reported that as high as 70 percent of the variation in obesity-related phenotypes is heritable in humans[4,5] which lead to tremendous efforts on understanding genetic influences on obesity.

G proteins, heterotrimeric guanine nucleotide-binding proteins, are ubiquitously expressed in human cells and consist of alpha, beta, and gamma subunits.[6] G proteins are in charge of regulating intracellular signal transduction in the G protein coupled receptors (GPCR) activation and play a crucial role in diverse physiological processes such as metabolism, satiety, cardiovascular functions, and immune response.[7,8] Therefore, dysregulation of the G-proteins and GPCR is associated with various diseases such as Alzheimer's disease,[9] hypertension,[10] cancers[11] and endocrine related diseases.[12] In particular, the genetic sequences of G protein beta3 subunit (GNB3) gene are known to be highly

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†To whom correspondence should be addressed.
tel: +82-2-920-7211, e-mail: mlee@sungshin.ac.kr

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polymorphic, so that multiple genetic polymorphisms appear due to its wide array of physiological roles.[13] Among these polymorphisms, the GNB3 C825T polymorphism has been intensively studied. The GNB3 C825T polymorphism, located in exon 10 of GNB3, has been shown to enhance the activation of heterotrimeric G protein *in vitro*[14] and has been shown to be variably associated with hypertension and obesity.[14-16]

The association of the GNB3 C825T polymorphism with obesity was reported in young German, Chinese, African Black, and Caucasian individuals,[15,17-20] although the allelic frequencies and frequencies of major haplotypes of the GNB3 C825T polymorphism differ by race and sex.[21-23] It has reported that the 825T allele may be associated with an increased risk for hypertension and obesity in countries with a Westernized lifestyle.[21,22] A recent study on Korean obese women demonstrated that the GNB3 825T allele is associated with greater visceral fat[24] and differentially affects body fat loss by sibutramine treatment of the female obese patients.[25] Thus, in countries with a Westernized lifestyle including South Korea, the prevalence of GNB3 825T allele might be a useful tool to predict an obesity and hypertension epidemic. However, the potential interaction of the GNB3 C825T polymorphism with dietary intake, obesity and metabolic syndrome risk factors in Korean children has not been closely evaluated yet.

A low level of HDL is one of the five criteria for the diagnosis of metabolic syndrome.[26] Furthermore, the negative relationship between HDL and the risk of developing ischemic heart disease is well accepted.[27,28] However, all HDL is not created equal; among HDL subfractions, the HDL_{2a}, HDL_{2b}, and HDL_{2c} lipoproteins represent cardio-protective subfractions, and HDL_{3b} is associated with increased cardiovascular risk.[28,29] In addition, our previous study demonstrated that small-sized HDL, HDL_{3b} was associated with increased metabolic syndrome risk factors and closely related to waist circumference (WC), BP, triglyceride (TG), and HOMA-IR in Korean adults.[30]

The objective of the present study was to investigate the potential interaction of the GNB3 C825T polymorphism, a risk factor for chronic disease in various ethnicities, with obesogenic environments, such as dietary intake, lipid profiles and insulin resistance-related factors, in overweight/obese Korean children.

METHODS

Study population

The study participants were recruited from 3rd grade elementary school, aged 8 to 9 years old, in seven elementary schools located in Guro-gu, Seoul, South Korea (Total n = 1,260; 50.3% boys; 49.7% girls) from April 2007 to May 2008. Informed assent and consent were obtained from the children and parents in accordance with the ethical standards of the responsible committee on human experiments. The study was approved and regulated by the Institutional Review Board of Korea University, Guro hospital (#:GR0837-001). Experimental research design of the present study is shown in Fig. 1.

Anthropometric, blood pressure and biochemical parameters

Height, weight, and WC were measured using standardized techniques with calibrated equipment. BMI was calculated as weight/height^2 (kg/m^2). Gender-specific BMI-for-age in the 2007 Korean National Growth Chart was used to formulate the following categories: underweight was defined as a BMI of lower than 5th percentile, normal weight was defined as a BMI of equal to or higher than 5th percentile lower than the 85th percentile, overweight was defined as a BMI of equal to or higher than the 85th percentile and lower than the 95th percentile, and obese was defined as a BMI of equal to or higher than the 95th percentile of the gender-specific BMI for age in the growth chart.[31,32] In the present study, the obese group (OB) included the overweight and obese subjects, and the normal weight group (NOR) included the normal and underweight subjects. BP was measured using a sphygmomanometer (Baum Co., Inc., South Korea). The subjects were allowed to rest more than 5 min, and subject's arm was horizontally positioned to measure systolic blood pressure (SBP) and diastolic blood pressure (DBP). Blood samples were collected in the morning after the subjects had fasted for 12 hr. Serum was separated by centrifugation and stored at -70°C until analysis. Total cholesterol (TC), HDL-c and TG concentrations were measured using a Hitachi-7600 analyzer (Hitachi Ltd., Japan), and LDL-c was calculated by the Friedewald formula as previously described.[33] Fasting blood insulin levels were measured using ECLIA (Electro-chemiluminescence immunoassay) and detected by automated immunology analyzer Elecsys 2010 (Roche Diagnostic, UK).

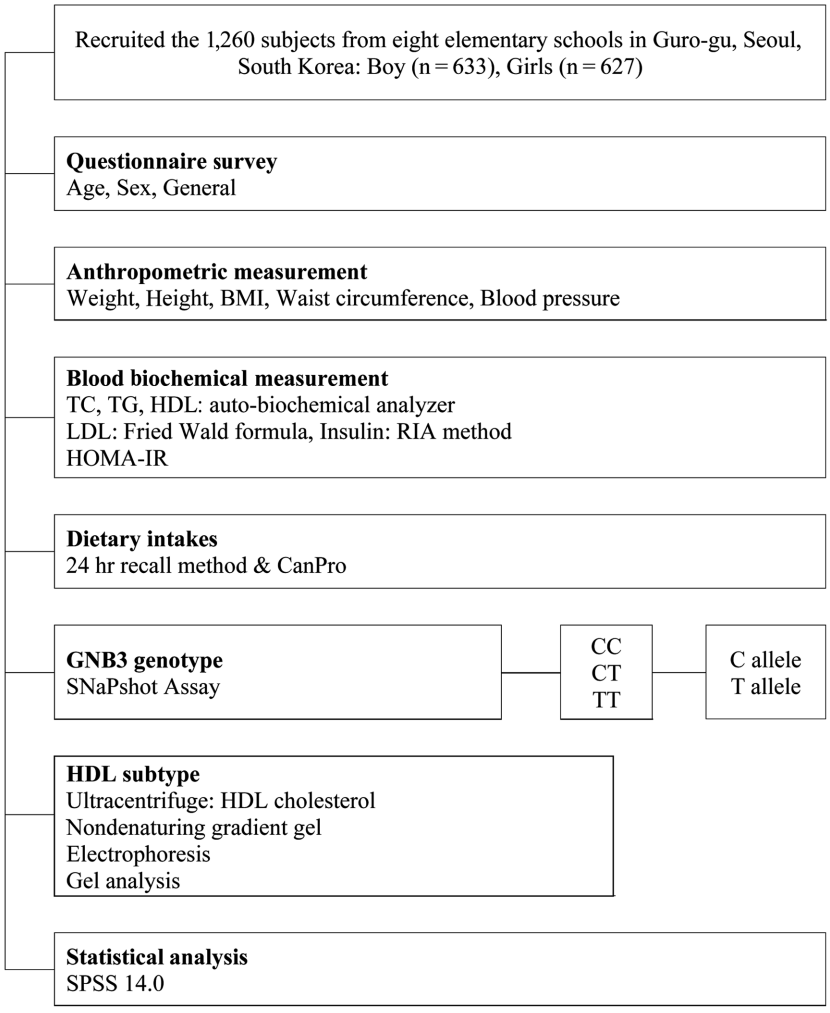

Fig. 1. Experimental research design

GNB3 gene polymorphism analysis

The GNB3 gene polymorphism was analyzed using a SNaPshot® multiplex kit according to the manufacturer's instruction. Briefly, genomic DNA was extracted using the Labo Pass™ Blood Mini Kit (Cosmo Genetech, Seoul, South Korea) and stored at -70°C until analysis. The primers used for GNB3 polymorphism analysis were as follows: 5'-GGAGCTGAGAATTGCTGTAG-3 (forward) and 5'-TGTAAAACGACGGCCAGT-3 (reverse). Ten nanograms of DNA was used in the reaction mixture containing 0.5 pM forward/reverse primer, 1 μL of 10X PCR buffer, 250 μM dNTP, and 0.25 units of DNA Taq polymerase. The thermocycling procedure consisted of pre-denaturation at 95°C for 10 min and 35 cycles of denaturation at 95°C for 30 sec, annealing at 72°C for 1 min, and extension at 72°C for 10 min. The amplification was performed using PCR machine (GeneAmp® PCR system 9700, Applied Biosystems, USA), and the results were analyzed by an ABI Prism® 3730*xl*DNA Analyzer and GeneMapper4.0 analysis software (Applied Biosystems, USA).

HDL particle size and its subfraction analysis

Samples (Total n = 60; 45% boy; 55% girls) from randomly selected subjects were used to analyze HDL particle size and subfraction. Plasma was obtained by centrifugation, and sequential preparation was then performed to collect the HDL fraction ($d \leqq 1.21$ mg/ml) by ultracentrifugation (Hitachi CS150GXL, Japan). The HDL fraction was analyzed by nondenaturing gradient gel electrophoresis, as described previously.[30,34] Briefly, the electrophoresis buffer contains 90 mM Tris, 80 mM boric acid, and 3 mM EDTA. Prior to the run, the electrophoresis system was pre-run for 20 min at

80 V. Twelve microliters of a sample mix (sample : sample buffer (40% sucrose, 0.01% bromophenyl blue), 2:1) was loaded, and electrophoresis was performed at 100 V for 2 hr, 130 V for 4 hr, 150 V for 18 hr, and 120 V for 2 hr. The gel was fixed with 10% sulfosalicylic acid for 30 min and stained with 0.1% Coomassie G-250 for 1.5 hr. The stained gel was destained by destaining solution containing 7.5% acetic acid and 5% methanol and was then stored in 1% acetic acid solution at 4°C. The Coomassie Blue G-250-stained gels were analyzed with Image-Master ID software 4.0 (Amersham Pharmacia Biotech, USA).

Assessment of nutrient intake

The parents of the subjects were asked to provide 3-day dietary records (2 weekdays and 1 weekend day). Nutrient analyses of these dietary records were conducted using CAN-Pro 3.0 software (The Korean Nutrition Society, Korea).[31] Nutrient intake data were adjusted by energy and evaluated based on the Dietary Reference Intakes for Koreans (KDRI).

Statistical analysis

The data are presented as the mean ± SD. To control for total energy intake, all nutrients were adjusted for total energy intake by using the residual method.[35] A x^2-test was used to analyze the allele of GNB3 and its genotype distribution. The mean differences were analyzed by t-tests or analysis of variance (ANOVA). Partial Pearson's correlation coefficients (r) were calculated and x^2-tests were performed to determine the relationships among variables. Significance was set at $p \leq 0.05$. Statistical analysis was performed using SPSS (14.0) for Windows (SPSS Inc., IL, USA).

RESULTS

General participant characteristics

BMI, WC, and SBP were significantly higher in boys than in girls; there was no difference in DBP. TC, TG and LDL-c levels were significantly lower in boys than in girls, but the HDL-c was significantly higher in boys than in girls (Table 1). There were no differences in insulin resistance-related factors and HDL peak size by gender. Boys appeared to have significantly higher HDL_{2a} subfractions and significantly lower HDL_{3c} subfractions than girls. Energy and nutrient intake, including carbohydrate, protein, fat, sodium and potassium, were significantly higher in boys than in girls. The anthropometrics and metabolic syndrome risk factors, including the lipid profile except for HDL and insulin resistance related factors, were significantly higher in the OB group compared to the NOR group for both genders. The HDL peak size was smaller in the OB group than in the NOR group. The HDL_{2b} subfraction was more decreased in the OB group than in the NOR group, but the HDL_{3c} subfraction was more increased in the OB group than in the NOR group. Energy intake in the OB group was increased mainly due to higher protein intake compared to the NOR group. In girls in the OB group, an increase in fat intake also contributed to the higher energy intake. Sodium and potassium intake were significantly higher in boys than in girls; however, the difference was not observed between the NOR and OB groups for both genders.

Gender difference of anthropometrics, BP, lipid profile, and nutrient intake by GNB3 polymorphism

The genotype frequencies of the GNB3 polymorphism were CC = 23.7% (n = 290), CT = 52.1% (n = 639), and TT = 24.2% (n = 297), and the allele frequencies were C allele = 49.7% (n = 1,219) and T allele = 50.3% (n = 1,233) in the subjects of the present study. There were no significant differences according to gender in the allelic frequencies in obese and normal weight subjects. Boys with the T allele had higher BMI, WC, SBP and TG but lower HDL than boys with the C allele (Table 2). Additionally, the boys with the T allele had significantly higher calcium and phosphorus intake with no significant differences in nutrient sources for energy than the boys with the C allele. Girls with the T allele had lower BMI and WC but higher energy intake and SBP than the girls with the C allele. Inconsistently with the lower BMI and WC in the girls with the T allele, these girls appeared to have higher energy intake than the girls with the C allele, with no significant differences in energy source nutrients or vitamins and minerals.

Characteristics of the subjects by gender and BMI according to the GNB3 polymorphism

We next determined whether the GNB3 C825T polymorphism further influences the anthropometrics, lipids and insulin resistance-related factors in boys and girls in the NOR and OB groups (Fig. 2). Regarding the BMI and WC, there was no significant difference between the boys with the C allele and the boys with the T allele in both the NOR group and the OB group. However, the girls with the T allele had significantly lower BMI and WC than the girls with the C

Table 1. Subject characteristics according to gender and BMI

Characteristics	Total			P-value[2]	Boys (n = 633)		Girls (n = 627)		p-value[3]	p-value[4]
	Total (n = 1,260)	Boys (n = 633)	Girls (n = 627)		NOR (n = 522)	OB[6] (n = 111)	NOR (n = 550)	OB (n = 76)		
Anthropometrics										
BMI (kg/m^2)	17.8 ± 0.1[1]	18.2 ± 0.1	17.4 ± 0.1	0.000	17.2 ± 1.8^b	23.2 ± 2.4^d	16.7 ± 1.7^a	21.7 ± 1.7^c	0.000	0.000
WC (cm)	59.2 ± 0.1	60.7 ± 0.2	57.6 ± 0.2	0.000	58.3 ± 5.7^b	71.9 ± 7.3^d	55.9 ± 5.0^a	67.2 ± 4.7^c		
Blood pressure										
SBP (mmHg)	109.3 ± 0.3	110.5 ± 0.5	108.1 ± 0.5	0.000	108.5 ± 15.6^a	120.3 ± 19.1^c	106.7 ± 15.7^a	115.3 ± 18.9^b	0.000	0.000
DBP(mmHg)	70.1 ± 0.3	70.4 ± 0.4	69.7 ± 0.4	NS	69.1 ± 13.0^a	76.9 ± 14.0^c	68.7 ± 12.9^a	74.6 ± 13.6^b	0.000	0.000
Lipid profiles									0.000	0.000
TC (mg/dL)	175.3 ± 0.6	173.7 ± 0.9	177.0 ± 0.9	0.009	172 ± 30.9^a	178.7 ± 30.4^b	175.6 ± 30.8^{ab}	187.1 ± 30.6^c	0.000	0.000
TG (mg/dL)	75.6 ± 0.8	70.1 ± 1.2	81.1 ± 1.2	0.000	65.8 ± 32.7^a	90.5 ± 54.7^c	76.6 ± 37.4^b	79.3 ± 10.9^d	0.000	0.000
HDL-c (mg/dL)	56.1 ± 0.2	56.9 ± 0.3	55.3 ± 0.3	0.000	58.0 ± 10.3^d	52.4 ± 9.5^b	56.0 ± 10.3^c	50.5 ± 8.1^a	0.000	0.000
LDL-c (mg/dL)	104.0 ± 0.6	102.9 ± 0.8	105.2 ± 0.8	0.042	101.1 ± 28.3^a	108.2 ± 28.0^b	104.0 ± 28.6^{ab}	115.5 ± 27.6^c	0.000	0.000
Insulin resistance related factors										
FBS (mg/dL)	77.7 ± 0.2	78.0 ± 0.3	77.3 ± 0.3	NS	77.6 ± 11.3^a	80.9 ± 14.7^b	76.8 ± 9.9^a	79.3 ± 10.9^b	0.000	0.000
Insulin (nU/mL)	7.3 ± 0.1	7.1 ± 0.2	7.4 ± 0.2	NS	6.7 ± 6.5^a	9.4 ± 6.4^b	6.7 ± 5.4^a	11.2 ± 9.4^c	0.000	0.000
HOMA-IR	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	NS	1.3 ± 1.4^a	1.9 ± 1.4^b	1.3 ± 1.1^a	2.2 ± 1.9^c	0.000	0.000
HDL subfractions n = 60		n = 27	n = 33			n = 12	n = 22	n = 11		
Peak size (nm)	9.7 ± 1.1	9.8 ± 1.0	9.5 ± 1.2	NS	10.4 ± 0.2^b	9.1 ± 0.3^{a*}	9.9 ± 0.3^b	8.9 ± 0.3^{a*}	0.000	0.002
HDL_{2b} (%)	35.2 ± 3.4	35.5 ± 3.2	34.9 ± 3.6	NS	36.5 ± 0.9	34.3 ± 1.0*	35.5 ± 0.8	33.8 ± 1.0*	0.032	NS
HDL_{2a} (%)	20.7 ± 1.1	21.1 ± 1.2	20.4 ± 1.1	0.021	21.0 ± 0.3^b	21.2 ± 0.4^b	20.6 ± 0.2^{ab}	20.1 ± 0.3^a	NS	0.038
HDL_{3a} (%)	16.7 ± 1.1	16.9 ± 1.1	16.5 ± 1.0	NS	16.6 ± 0.3	17.1 ± 0.3	16.5 ± 0.2	16.4 ± 0.3	NS	NS
HDL_{3b} (%)	11.8 ± 1.1	11.6 ± 1.2	11.9 ± 1.0	NS	11.4 ± 0.3	11.9 ± 0.4	11.7 ± 0.2	12.2 ± 0.3	NS	NS
HDL_{3c} (%)	15.7 ± 2.5	14.9 ± 2.2	16.3 ± 2.5	0.024	14.4 ± 0.5^a	15.5 ± 0.6^{b*}	15.7 ± 0.5^b	17.5 ± 0.7^{c*}	0.001	0.003
Energy and nutrient intake										
Energy (kcal)	1,617 ± 380.7	1,698.4 ± 387.7	1,536.2 ± 355.6	0.000	1,690 ± 391.9^b	1,733.7 ± 367.5^{b*}	1,532.5 ± 360.5^a	1,558.0 ± 325.2^{a*}	0.02	0.000
Carbohydrate (g)	220.8 ± 24.4	230.7 ± 23.2	210.8 ± 21.2	0.000	231.2 ± 23.2^c	228.5 ± 23.5^c	211.5 ± 20.7^b	207.1 ± 23.7^a	NS	0.000
Protein (g)	33.6 ± 13.9	69.7 ± 8.8	62.8 ± 16.9	0.000	69.5 ± 8.8^c	70.8 ± 8.8^{c*}	62.2 ± 9.2^b	65.1 ± 11.7^{a*}	0.000	0.000
Fat (g)	54.4 ± 9.7	57.6 ± 9.5	51.2 ± 8.9	0.000	57.6 ± 9.4^b	57.6 ± 9.8^b	51.5 ± 8.8^a	52.7 ± 9.0^{a*}	0.046	0.000
Na (mg)	3,573.6 ± 785.0	3,714.2 ± 791.5	3,431.6 ± 752.3	0.000	3,710.7 ± 776.1^b	3,729.5 ± 857.2^b	3,422.2 ± 751.2^a	3,486.6 ± 759.0^a	NS	0.000
K (mg)	2,338.3 ± 484.8	2,452.0 ± 500.9	2,223.6 ± 439.3	0.000	2,464.1 ± 484.0^b	2,408.0 ± 568.0^b	2,217.9 ± 436.9^a	2,257.1 ± 452.7^a	NS	0.000

1) Data are expressed as the mean ± SD.; adjusted by energy 2) P-value: t-test between boys and girls 3) p-value: t-test between NOR and OB, expressed by an asterisk 4) p-value: ANOVA, expressed by a lowercase letter

allele in both the NOR and OB groups. Although there were no significant differences in the anthropometrics related to the GNB3 allele in boys in the OB group, the boys with the T allele in the OB group had higher TC levels than the boys with the C allele in the OB group. The girls with the T allele in the OB group appeared to have significantly higher TC levels than the girls with the C allele, which did not reconcile with their lower BMI and WC. Regardless the GNB3 allele, HOMA-IR levels were higher in boys in the OB group than in boys in the NOR group. The HOMA-IR levels were significantly lower in the girls with the T allele than the girls with the C allele in the OB group in accordance with the lower BMI and WC in the girls with the T allele. Thus, there was a clear gender difference of GNB3 polymorphism in obese children.

Characteristics of HDL subfractions and insulin resistance related factors by HDL peak size

We observed that there were significant alterations in HDL-c levels and HDL subfractions by gender and BMI in the Korean children in the present study. To investigate the relationship between HDL peak size and HDL subfractions, as well as insulin resistance related factors in this population, the HDL peak size was divided into quartiles (1st quartile (n = 16); ≤ 8.80, 2nd quartile (n = 18); 8.81~9.18, 3rd quartile (n = 21); 9.80~10.60, 4th quartile (n = 14); ≥ 10.61). The percentage of the HDL_{2b} subfraction increased as the HDL peak size increased, but the percentage of the HDL_{3c} subfraction decreased as the HDL peak size decreased (Fig. 3, A and B). Additionally, fasting insulin levels and HOMA-IR tended to decrease with an increase in the HDL

Table 2. Differences in anthropometrics, blood pressure, lipid profiles and nutrients intake by gender and GNB3 alleles

GNB3	Total (n = 2,452)		Boys (n = 1,236)		Girls (n = 1,216)		p-value [3]
	C (n = 1,219)	T (n = 1,233)	C (n = 604)	T (n = 632)	C (n = 615)	T (n = 601)	
Anthropometric measurement							
BMI (kg/m^2)	17.8 ± 0.1[1]	17.9 ± 0.1	18.1 ± 0.1^c	18.4 ± 0.1^{d*}[2]	17.6 ± 0.1^b	17.2 ± 0.1^{a*}	0.000
WC (cm)	59.1 ± 0.2	59.2 ± 0.2	60.3 ± 0.3^c	61.2 ± 0.3^{d*}	57.9 ± 0.3^b	57.0 ± 0.3^{a*}	0.000
Blood pressure							
SBP (mmHg)	109.0 ± 0.5	109.7 ± 0.5	110.1 ± 0.7^c	111.4 ± 0.7^d	107.9 ± 0.7^a	108.0 ± 0.7^b	0.002
DBP (mmHg)	69.7 ± 0.4	70.4 ± 0.4	70.1 ± 0.6	71.2 ± 0.5	69.4 ± 0.5	69.6 ± 0.5	NS
Lipid profile							
TC (mg/dL)	174.8 ± 0.9	175.6 ± 0.9	173.2 ± 1.3	174.2 ± 1.2	176.4 ± 1.2	177.2 ± 1.3	NS
TG (mg/dL)	74.8 ± 1.2	76.3 ± 1.2	69.6 ± 1.6^a	71.0 ± 1.5^{a*}	79.4 ± 1.7^b	81.7 ± 1.7^b	0.000
HDL-c (mg/dL)	56.1 ± 0.3	56.2 ± 0.3	57.3 ± 0.4^b	56.9 ± 0.4^{b*}	54.0 ± 0.4^a	55.5 ± 0.4^a	0.000
LDL-c (mg/dL)	103.7 ± 0.8	104.2 ± 0.8	102.0 ± 1.2	103.2 ± 1.1	105.3 ± 1.2	105.5 ± 0.4	NS
Insulin resistance related profiles							
FBS (mg/dL)	77.4 ± 0.3	77.7 ± 0.3	77.6 ± 0.5	78.5 ± 0.5	77.1 ± 0.4	77.0 ± 0.4	NS
Insulin (nU/mL)	7.4 ± 0.2	7.1 ± 0.2	7.1 ± 0.3	7.2 ± 0.3	7.6 ± 0.3	7.1 ± 0.3	NS
HOMA-IR	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	NS
Energy and Nutrient intake							
Energy (kcal)	1,614.0 ± 394.3	1,620.1 ± 367.6	1,713.2 ± 406.1^c	1,677.9 ± 369.8^c	1,516.7 ± 356.8^a	1,559.5 ± 355.6^{b*}	0.000
Carbohydrate (g)	221.1 ± 24.2	220.6 ± 24.4	231.3 ± 22.7^b	230.2 ± 23.1^b	211.2 ± 21.3^a	210.6 ± 21.4^a	0.000
Protein (g)	65.8 ± 14.2	66.8 ± 13.6	69.4 ± 9.0^b	69.9 ± 8.7^b	62.8 ± 9.9^a	62.4 ± 9.5^a	0.000
Fat (g)	54.2 ± 9.5	54.5 ± 9.9	57.4 ± 8.9^b	57.7 ± 9.7^b	51.6 ± 9.0^a	51.7 ± 8.8^a	0.000
Na (mg)	3,576.9 ± 794.7	3,566.9 ± 782.7	3,707.4 ± 805.4^b	3,715.9 ± 786.7^b	3,448.8 ± 763.1^a	3,410.1 ± 747.7^a	0.000
Ca (mg)	565.8 ± 162.0	578.3 ± 168.8	588.5 ± 168.6^c	612.5 ± 165.0^{d*}	544.6 ± 151.6^a	543.5 ± 164.9^a	0.000
P (mg)	958.4 ± 158.0	972.9 ± 160.3[*2]	1,005.1 ± 164.7^c	1,025.5 ± 155.6^{d*}	912.5 ± 136.3^a	917.5 ± 145.9^a	0.000
Vitamin A (μg R.E)	828.9 ± 329.3	833.7 ± 320.9	855.4 ± 330.7^b	863.4 ± 315.1^b	802.8 ± 326.0^a	802.4 ± 324.2^a	0.000
Vitamin B_1 (mg)	1.6 ± 0.4	1.6 ± 0.4	1.7 ± 0.3^b	1.7 ± 0.3^b	1.6 ± 0.4^a	1.6 ± 0.4^a	0.000
Vitamin B_2 (mg)	1.8 ± 0.3	1.8 ± 0.3	1.8 ± 0.3^b	1.8 ± 0.3^b	1.7 ± 0.3^a	1.7 ± 0.3^a	0.000

1) Data are represented as the mean ± SSD. adjusted by energy 2) *: P-value<0.05, t-test between C allele and T allele, expressed by an asterisk 3) p-value: ANOVA among 4 groups in boys and girls, expressed by a lowercase letter

peak size (Fig. 3C and D).

DISCUSSION

This was the first international report on the gender-specific interaction of the GNB3 C825T polymorphism with obesogenic environments, such as dietary intake, lipid profiles and insulin resistance-related factors, in obesity of Korean children aged between 8 and 9 years. The frequencies of the 825C and 825T alleles in the samples were 0.497 and 0.503, respectively, which is in agreement with the previously reported values in a Korean adult population.[23] The frequencies were 0.237, 0.521, and 0.242 for the 825C/C, 825T/C, and 825T/T genotypes in the overall study sample, respectively. Compared to the worldwide ethnic distribution of the GNB3 825T allele results for South Koreans (n = 31) by Siffert et al.,[21] our result appeared to have lower frequencies of the 825C allele (0.497 vs. 0.560) and higher frequencies of the 825T allele (0.503 vs. 0.440). It is well recognized that the allelic frequencies of the GNB3 C825T polymorphism and frequencies of major haplotypes differ by race[21-23] and sex.[24,36]

In the present study, boys were significantly heavier than girls based on BMI and WC, partially due to higher energy intake by all three nutrients for energy supply. Although the boys were significantly heavier, the TC, TG, and LDL-c levels were significantly lower than those in girls suggesting Korean boys in the current study are metabolically healthy, although the average BMI was significantly higher than that of girls. This discrepancy of BMI and lipid profile in both genders could be partially explained by different patterns of the HDL subfractions with significantly lower HDL_{2a} and higher HDL_{3c} in girls than those in boys (Table 1). In addition, our previous study in Korean adults aged average 49.2 ± 11.5 years demonstrated gender differences in large HDL_{2b} and small HDL_{3b}, HDL_{3c} subfractions.[30] On the other

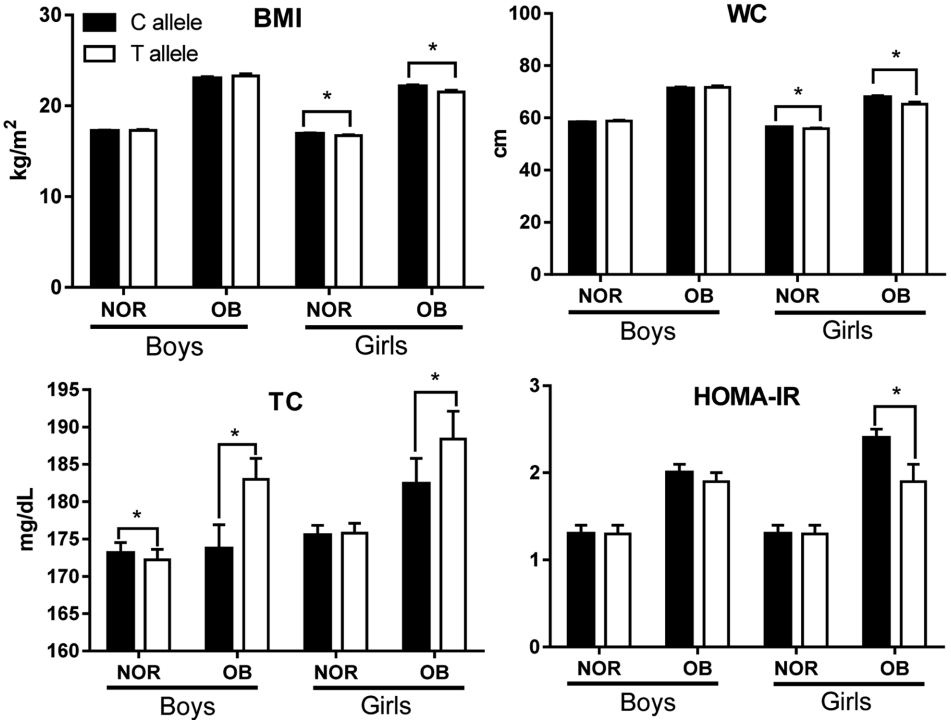

Fig. 2. Anthropometric measurement, lipid profile and insulin resistance-related factors in boys and girls by BMI and GNB3 alleles. Korean childhood obesity classification by BMI percentiles: obesity ≥ 95 percentile, 85 percentile ≤ overweight ≤ 95 percentile, normal weight (NOR) < 85 percentile from the Korean Society of Obesity. The overweight group was included in the obese group (OB). An asterisk (*) indicates the statistical significance with $p < 0.05$ between the C and T alleles.

hands, Gracés et al.[37] demonstrated that some metabolic consequences of obesity in Spanish obese children aged 6-8 year were similar to those found in adults such as elevated TG, insulin, and HOMA-IR, lower HDL-c, however some features such as blood glucose, TC, LDL-c behaved differently. Thus, it is worthwhile to note that the association of obesity with risk factors including elevated TG, TC, LDL-c, HDL-c, insulin and HOMA-IR may be altered by children age and depends on the chronology of sexual maturation.[37] The anthropometrics, BP, and metabolic syndrome risk factors including the lipid profile, except for HDL and insulin resistance related factors, were significantly higher in the OB group than in the NOR group for both genders. An inverse relationship between HDL particle diameter and TG level has been shown in a previous study,[38] and it was also observed in the present study by demonstrating significantly higher HDL particle size with lower TG levels in the NOR group than the OB group for both genders (Fig. 3).

Multiple studies have demonstrated the association of the GNB3 C825T polymorphism with obesity.[16,19-21,39] Thus it was proposed that the high number of GNB3 825T allele carriers could be a major determinant for the observed difference in the prevalence of obesity and obesity–related disorders in certain ethnic groups especially living in countries with uniform lifestyle conditions. On the other hands, a few studies did not confirm such an association between the C825T polymorphism and obesity.[40,41] A gender difference in the GNB3 polymorphism was shown in the present study, as evidenced by the boys with the T allele having a higher BMI than the boys with the C allele. There was no significant difference in BMI between the girls with the T allele and the girls with the C allele. A similar trend was observed in German male subjects aged between 58 and 59 years in which the TT genotypes were associated with higher BMI compared to the CC and CT genotypes.[36] Furthermore, the girls with the T allele consumed a significantly higher energy intake than the girls with the C allele without having different BMIs, whereas no energy intake difference was observed in the boys.

SUMMARY

The allelic differences in boys in the NOR and OB groups did not appear in the anthropometrics, whereas the allelic

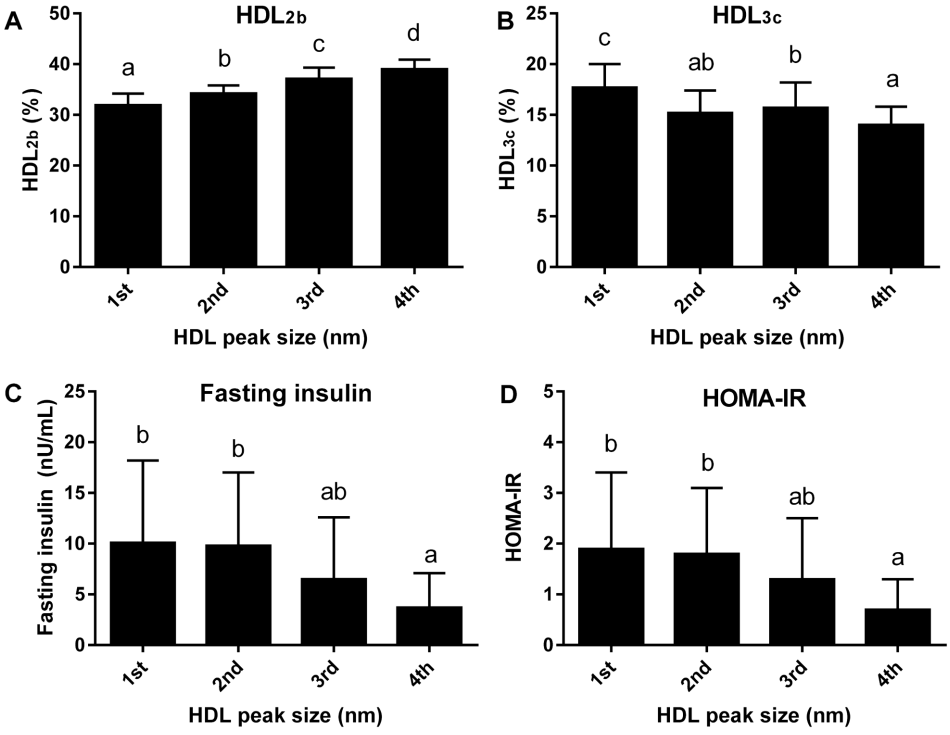

Fig. 3. Characteristics of HDL subfractions and insulin resistance related factors by HDL peak size. Data are represented as the mean ± SD (error bars) adjusted by energy; 1st quartile (n = 16); ≤ 8.80, 2nd quartile (n = 18); 8.81~9.18, 3rd quartile (n = 21); 9.80~10.60, 4th quartile (n = 14); ≥ 10.61. The significance differences were analyzed by ANOVA ($p < 0.05$) and are expressed as a lowercase letter.

differences of BMI and WC included lower levels in the girls with the T alleles in both the NOR and OB groups. The allelic differences in TC and LDL levels were displayed differentially depending on the BMI status of the boys. The limitations of the study included the following; (1) it was difficult to collect data for 3 days of 24-h diet recalls by students aged 8~9 years and (2) a lack of blood samples from subjects limited the number of samples to perform HDL subfraction analysis as well as a high cost of performing the analysis. Nevertheless, we believe this study is the first to show gender-specific interaction of the GNB3 C825T polymorphism with obesogenic environments, such as dietary intake, lipid profiles and insulin resistance-related factors, in obesity of Korean children aged between 8 and 9 years. Unlike a higher risk for negative health outcomes by the GNB3 polymorphism in various ethnicities, GNB3 polymorphism did not influence the obesogenic environments in overweight/obese Korean children. Finally, the high prevalence of the GNB3 T allele may not be a useful tool to predict an obesity and hypertension epidemic in Korean children.

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