Gender Differences in the Alteration of Obesogenic Environments in Korean Children According to GNB3 Polymorphism

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Abstract

Background: A single-nucleotide polymorphism, C825T in the G protein β3 subunit (GNB3) gene, is associated with essential hypertension and obesity. However, the potential interaction of the GNB3C825T polymorphism with obesogenic environments in obesity of Korean children has not been closely evaluated yet.

Methods: We analyzed data collected from 635 boys and 627 girls aged 8-9 yearsrecruited from seven elementary schools in Seoul, South Korea. Measures of anthropometry and blood pressure (BP) as well as fasting blood samples were used in the analysis. Three-day food records were collected.

Results: Obese children had higher BP, lipid profiles and insulin resistance-related factors than did lean children. Higher body mass index (BMI), waist circumference (WC) and BP and lower plasma lipid profiles were observed in boys than in girls. The genotype frequencies of the GNB3 polymorphism were CC=23.7%, CT=52.1%, and TT=24.2%, and the allele frequencies were C allele=49.7% and T allele=50.3 in the subjects. There was no significant difference according to gender and obesity with allelic frequencies. Boys with the T allele were more obese than those with the C allele, while the C and T alleles, according to anthropometrics and plasma variables, did not differ between the obesity and lean groups. In contrast to boys, obese girls with the T allele had lower BMI and WC than those with the C allele, although they had higher plasma cholesterol and energy intakes compared to those with the C allele. We found that the HDL3c subtype, fasting insulin and homeostasis model assessment of insulin resistance decreased with increasing HDL peak amount, which was positively correlated with total HDL-cholesterol. However, the HDL subtypes were not changed by the GNB3 C825T polymorphism.

Conclusion: The GNB3 C825T polymorphism influences the childhood obesity rate and energy intakesdifferentially by gender in Korean children.

Abbreviations


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 to the etiology of obesity [3]. In fact, it was reported that as high as 70 percent of the variation in obesity-related phenotypes is inheritable in humans [4,5].

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 GNB3 gene are known to be highly 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 men, Chinese men, African Blacks, and Caucasians [15,17-20], although the allelic frequencies and...
immunoassay(IA) technique. Insulin was measured using a conventional RIA method (Linco Research, Inc., USA), and its homeostasis model assessment (HOMA-IR) was calculated [33]. HDL cholesterol, triglycerides (TG), and insulin resistance (HOMA-IR) were measured using the LaboPassTM Blood Mini Kit (Cosmo Genetech, Seoul, South Korea). Total cholesterol (TC), HDL-c and TG concentrations were measured using a Hitachi-7600 analyzer (Hitachi Ltd., Japan), and LDL-cholesterol (LDL-c) was calculated by the Fried wald formula as previously described [33]. Fasting blood insulin levels were measured using ECLIA (Electrochemiluminescence immunoassay) and detected by automated immunology analyzer Elecsys 2010 (Roche Diagnostic, UK).

**GNB3 gene polymorphism analysis**

The GNB3 gene polymorphism was analyzed using an SNPPhat® multiplex kit according to the manufacturer's instruction. Briefly, genomic DNA was extracted using the LaboPass™ 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'-GGAGCTGAGATTTGCTGTAG-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, 250μM 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® 3730xl DNAAnalyzer and GeneMapper 4.0 analysis software (Applied Biosystems, USA).

**HDL particle size and subfraction analysis**

Samples (Total = 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 (ds<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 electrophoresis, the electrophoresis system was pre-run for 20 min at 80 V. Twelve microliters of serum were separated by centrifugation and stored at 7°C until electrophoresis. Ten microliters of sample were loaded onto gel and electrophoresis was performed at 100 V for 2 hr, 130 V for 4 hr, 150 V for 18 hr, and 120 V for 10 min.
A 4\textsuperscript{th}-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 \chi\textsuperscript{2}-tests were performed to determine the relationships among variables. Significance was set at P ≤ 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\textsubscript{a} subfractions and significantly lower HDL\textsubscript{b} 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\textsubscript{a} subfraction was more decreased in the OB group than in the NOR group, but the HDL\textsubscript{b} 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\%, CT=52.1\%, and TT=24.2\%, and the allele frequencies were C allele=49.7\% and T allele=50.3\% 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, BP, lipids and insulin resistance-related factors in boys and girls in the NOR and OB groups (Figure 2). Regarding the BMI, WC, and BP, 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 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.

Figure 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. All significance levels were P<0.01 in the four groups in both the boys and girls (not described in the above four graphs).
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 HDL2b subfraction increased as the HDL peak size increased, but the percentage of the HDL3a subfraction decreased as the HDL peak size decreased (Figure 3 A and 3B). Additionally, fasting insulin levels and HOMA-IR tended to decrease with an increase in the HDL peak size (Figure 3C and 3D). Furthermore, the HDL peak size and the distribution of HDL subfractions were analyzed based on the GNB3 allele, and there was no significant difference in HDL peak size and HDL subfractions by GNB3 polymorphism in both genders (data not shown). However, we found that the HDL peak size was positively correlated with total HDL-c (r=0.522, p<0.01) whereas the HDL peak size was negatively correlated with BMI (r=-0.449, p<0.01), WC (r=-0.337, p<0.01), and TG (r=-0.349, p<0.01) in our study population (data not shown).

Figure 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 lower case letter.

Discussion

This was the first international report on the gender-specific interaction of the GNB3 C825T polymorphism with obeseogenic 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 [33]. 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 HDL2a and higher HDL3a in girls than in those boys. In addition, our previous study in Korean adults aged average 49.2±11.5 years demonstrated gender differences in large HDL2b and small HDL3a, HDL subfractions [30]. On the other hand, Graciet et al. [37] demonstrated that some metabolic consequences of obesity in Spanish obese children aged 6-8 years 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.

Multiple studies have demonstrated the association of the GNB3 C825T polymorphism with obesity [16,19-21,39]. However, 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.

The allelic differences in boys in the NOR and OB groups did not appear in the anthropometrics, whereas the allelic 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. We also demonstrated a positive relationship between HDL2b subfraction and HDL peak size and inverse relationships between HDL3a subfraction, insulin and HOMA-IR by HDL peak size in Korean children aged between 8 and 9 years of age. Furthermore, the HDL peak size and the distribution of HDL subfractions depending on the GNB3 alleles did not significantly differ with HDL peak size and HDL subfractions according to GNB3 polymorphism in both genders.
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. In conclusion, the results of the present study showed that the boys tended to be heavier than the girls in terms of BMI and WC, although lipid profiles were more favorable compared to those of the girls. Moreover, the T allele of GNB3 C825T was associated with the characteristics of the obesity-related phenotype, including increased BMI, WC, SBP, DBP, and TG, as well as decreased HDL-c, compared to the C allele only in the boys.

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