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Polymorphism of DsbA-L Gene Associates with Insulin Secretion and Body Fat Distribution in Chinese Population

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Abstract. Disulfide-bond-A oxidoreductase-like protein (DsbA-L) has been suggested to take part in the disulfide bond formation progress of proteins, including insulin and adiponectin. Recent study has demonstrated that expression of DsbA-L was decreased in obese mice and human subject, indicating that DsbA-L might be a potential target for treatment of metabolic diseases. We investigated the association of SNP-1308G/T (rs1917760) of DsbA-L gene with metabolic diseases. 589 normal glucose tolerance (NGT) subjects and 556 type 2 diabetes (T2DM) subjects were recruited. Each group was divided into normal weight (NW) (BMI<24 kg/m^2) subgroup and overweight/obesity (OW/OB) (BMI≥24 kg/m^2) subgroup. Genotype distributions and allele frequencies of SNP (-1308G/T) in DsbA-L gene were not associated with T2DM and obesity. However, it was observed that *T* allele carriers had better insulin secretion function compared with non-*T* allele carriers in NGT-NW group, not only the first phase insulin secretion (P=0.007) but also the second phase insulin secretion (P=0.031). Multiple linear regression analysis revealed that SNP-1308G/T polymorphism (rs1917760) was independently correlated with both first and second phase insulin secretion in NGT-NW group (R^2=0.055, P=0.007; R^2=0.029, P=0.041). Otherwise, *T* carriers had more visceral fat than non-*T* carriers (P=0.020) in NGT-OW/OB group. In conclusion, the SNP-1308G/T (rs1917760) genotypes of DsbA-L gene might participate in insulin secretion and body fat distribution. It is possible that polymorphisms of DsbA-L gene associated with metabolic diseases.

Key words: Disulfide-bond-A oxidoreductase-like protein (DsbA-L), single nucleotide polymorphism (SNP), insulin secretion, type 2 diabetes (T2DM)

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DISULFIDE-BOND-A OXIDOREDUCTASE (DsbA) family was a subfamily of the thioredoxin family and catalyzed disulfide bond formation during the folding of secreted proteins in bacterial [1, 2]. DsbA could increase the production of human proinsulin in the periplasmic space of *Escherichia coli* and it was a very effective partner for obtaining high amounts of soluble and correctly disulfide bridged proinsulin [3]. Disulfide-bond-A oxidoreductase-like protein (DsbA-L) is a renamed protein, which used to be called glutathione S-transferase-kappa (GST-kappa), shares high sequence and secondary structure homology to E. coli DsbA and has the same general folding as DsbA [4, 5, 6, 7]. Therefore, DsbA-L might also have the ability to catalyzed disulfide bond formation during protein assembling, such as insulin.

Recently, a study from Meilian Liu *et al*. has shown that DsbA-L was highly expressed in adipose tissue and specifically interacts with adiponectin which was an adipokine specifically secreted from adipose tissue and was strongly associated with the modulation of glucose and lipid metabolism in insulin sensitive tissues [4, 8]. Decreased expression of adiponectin was correlated with insulin resistance, obesity and type 2 diabetes [9, 10, 11, 12]. Overexpressing DsbA-L promoted adiponectin multimerization which character-

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ized by the formation of disulfide bonds, while suppressing DsbA-L expression markedly and selectively reduced adiponectin levels and secretion in 3T3-L1 adipocytes. DsbA-L expression level in adipose tissue was negatively correlated with obesity in mice and humans [4].

Therefore, DsbA-L might be a key regulator for both insulin and adiponectin biosynthesis and secretion. Dysfunction of DsbA-L might be a factor associated with both insulin biosynthesis or secretion and insulin resistance and metabolic diseases, such as type 2 diabetes (T2DM) and obesity.

Multiple mechanisms have been proposed to contribute to the pathogenesis of T2DM and obesity. A genetic etiology underlying the prevalence of these two metabolic diseases is widely accepted. Thus, we hypothesized that genetic variability in DsbA-L might play a role in the development of T2DM and obesity. DsbA-L gene spans a 5.68-kb region and locates at chromosome 7q34 [2]. Up to now, there is no report about the association of DsbA-L gene variation with metabolic diseases. In the present study, we investigated the association of variants in DsbA-L gene with glucose and lipid metabolism, serum insulin and adiponectin concentrations, and onset of T2DM and obesity in Chinese population.

Materials and methods

Experimental Subjects

All subjects were Chinese origin (Han Chinese) and lived in the same region at the time of the study. A total of 1145 subjects (603 male and 542 female) from Shanghai Diabetes Institute were recruited into this study. Among all the subjects, 589 had normal glucose tolerance (NGT) (age 54.92±11.68 yr; 264 male and 325 female), 556 had type 2 diabetes (T2DM) (age 52.29±16.66 yr; 339 male and 217 female). All the type 2 diabetes subjects were newly diagnosed according to the WHO criteria (1999). To some extent, obesity is considered as the early prophase of T2DM, so each group was divided into two subgroups, normal weight (NW) group (BMI<24 kg/m^2) and overweight/obesity (BMI≥24 kg/m^2) group on the basis of the guidelines for prevention and control of overweight and obesity in Chinese adults [13]. The study was approved by the human research ethics committee of the hospital, and informed consent was obtained from each of the participants.

Phenotype Measurements

After an overnight fasting, height, weight, waist circumference and blood pressure were measured, and a blood sample was drawn for biochemical measurements and DNA extraction. Each participant received a 75-g oral glucose tolerance test (OGTT). BMI was calculated as the weight in kilograms divided by the square of the height in meters. Systolic and diastolic blood pressures were measured in the seated position after five minutes of rest. Abdominal visceral fat area (VA) and subcutaneous fat area (SA) were measured by magnetic resonance imaging at the level of L4-L5. Each fat area was calculated as previously described [14]. Plasma glucose was determined by the glucose oxidase method. Total serum adiponectin and insulin concentrations were measured in duplicates by RIA (Linco Research, St. Charles, MO). Lipid profile (total serum cholesterol, HDL-cholesterol, LDL-cholesterol and serum triglycerides) was measured with an automatic clinical analyzer 7600-20 (Hitachi, Japan).

Gel filtration and quantification of High molecular weight (HMW) adiponectin

HMW adiponectin concentrations were measured in part of the subjects from each group (n=186). 10-15μl human serum was diluted to 1ml with PBS and fractionated through a Hi-load 16/60 Superdex™ 200 column (GE Healthcare), and eluted with PBS at the flow rate of 1 ml/min. Each 1ml fraction was collected and subjected to ELISA analysis for human adiponectin [15].

Evaluation of insulin resistance and insulin secretion

Insulin sensitivity was assessed by Cederholm index [16], which was calculated as follows:

ISI=m/MG/log (M-INS)

$m = [75{,}000 + (Gluc_0 - Gluc_{120}) \times 0.19 \times weight]/120$

$MG = (Gluc_0 + Gluc_{120})/2$

$M\text{-}INS = (INS_0 + INS_{120})/2$

INS: insulin concentration in pmol/L

Gluc: glucose concentration in mmol/L

Insulin secretion index was evaluated using Stumvoll formula [17].

Stumvoll formula:

First phase insulin secretion

$=2.503+6.476\times INS_0-126.5\times Gluc_{120}+0.954\times INS_{120}-239.3\times Gluc_0$

Second phase insulin secretion

$=393+1.163\times INS_0-40.72\times Gluc_{120}+0.313\times INS_{120}$

INS: insulin concentration in pmol/L

Gluc: glucose concentration in mg/dl

SNP genotyping

According to the NCBI database (http://www.ncbi.nlm.nih.gov) and Hapmap project (http://www.hapmap.org), we chose the SNP -1308G/T (rs1917760) of DsbA-L gene, which was the only SNP of this gene with a minor allele frequency higher than 5% in Asian population, to investigate the association of DsbA-L gene polymorphism with glucose or lipid metabolism in Chinese population.

Genomic DNA was extracted from peripheral whole blood leukocytes. Genotyping of the SNP-1308G/T (rs1917760) was performed by PCR-RFLP. Forward primers 5'-CAAGCAACAATAATAAGAGC-3' and reverse primer 5'-GGTAATGGGCAAGATGAA-3' were used for amplification of the SNP-1308G/T region (rs1917760). The polymerase chain reaction (PCR) conditions were as follows: predegeneration at 95℃ for 5 min; 30 cycles of denaturation at 95℃ for 1 min, annealing at 55 ℃ for 1 min, and extending at 72 ℃ for 1min. The thermal cycle were completed with 72 ℃ for 10 min (Gene Amp PCR System 9700, Applied Biosystem, USA). The 263 bp PCR products were digested with the *Rsa* I restriction enzyme (New England Biolabs, USA) at 37℃ for 2 hours. The digested products were separated by electrophoresis in 12% neutral polyacrylamide gel stained with ethidium bromide and visualized on a UV trans-illuminator (Bio-Rad, USA).

Statistical analysis

Statistical analysis was performed using the SAS 8.1. Genotype and allele frequency distributions were compared using the χ^2 test. Hardy-Weinberg equilibrium was computed to the expected genotype distribution. Logarithmic transformation was used to normalize the distributions of plasma glucose, serum insulin, serum adiponectin, serum triglycerides, and Cederholm index. Data were shown as the median values (25% Q1 and 75% Q3) for non-normal distribution variables or mean values ± SD for normal distribution variables. Due to the small population of *T/T* carriers for the polymorphism, we combined *T/T* carriers with *T/G* carriers to one group. Student's *t*-test was used to compare continuous variables; adjustment was performed by Covariance (ANCOVA) analysis. The associations of DsbA-L gene polymorphism with the relevant clinical parameters were analyzed by Pearson correlation and multiple stepwise regression. In both Pearson correlation and multiple stepwise regression analysis, *T/T+T/G* were delegated by 1 and *G/G* was delegated by 2. All reported *P* values were two-tailed, and *P* values <0.05 were considered statistically significant.

Results

Distribution of SNP-1308G/T (rs1917760) of DsbA-L gene

The SNP-1308G/T (rs1917760) is in the 5' upstream of DsbA-L gene. Genotype distributions of SNP-1308G/T (rs1917760) of DsbA-L gene in the total subjects were: 3.5% of *T/T*, 30.8% of *T/G*, and 65.7% of *G/G*, which were in Hardy-Weinberg equilibrium. *G* is the major allele (*G* frequency=81.1%). These frequencies were not significantly different from those announced on NCBI database.

Association of SNP in DsbA-L gene with type 2 diabetes and obesity

To assess whether SNP-1308G/T (rs1917760) of DsbA-L gene contributed to the onset of T2DM, 589 subjects with NGT and 556 with T2DM were genotyped. The genotype distributions of this SNP were summarized in Table 1. Our study indicated that genotype distributions and allele frequencies of SNP (-1308G→T) had no association with T2DM. Within the NGT group, there were no significant differences in genotype distributions and allele frequencies of this SNP between normal weight (NGT-NW, BMI<24 kg/m^2) subjects and overweight/obesity (NGT-OW/OB, BMI≥24 kg/m^2) subjects. Within the T2DM group, there was a statistical difference in genotype distributions (*P*=0.043) between the two subgroups (T2DM-NW vs. T2DM-OW/OB), however, the allele frequencies of this SNP in these two subgroups are similar.

Table 1. Genotype distributions of the SNP-1308G/T (rs1917760)

	n	*T/T*	*T/G*	*G/G*
NGT	589	3.56 (21)	29.88 (176)	66.55 (392)
NGT-NW	310	5.16 (16)	27.74 (86)	67.10 (208)
NGT-OW/OB	279	1.79 (5)	32.26 (90)	65.95 (184)
T2DM	556	3.42 (19)	31.83 (177)	64.75 (360)
T2DM-NW	222	4.50 (10)	26.13 (58)	69.37 (154)
T2DM-OW/OB	334	2.69 (9)	35.63 (119)	61.68 (206)

Data are percentage value of genotype distributions (n).
NGT, normal glucose tolerance; T2DM, type 2 diabetes; NW, normal weight; OW/OB, overweight or obese.

Table 2. Clinical characteristics, body fat distribution, insulin sensitivity and insulin secretion according to genotypes of the SNP-1308G/T (rs1917760)

	NGT		T2DM	
	T/T+T/G	*G/G*	*T/T+G/G*	*G/G*
Age(y)	53.69±17.14	51.59±16.39	55.35±11.09	54.68±12.00
Sex(M/F)	78/119	186/206	124/72	215/145
BMI(kg/m^2)	24.25±4.49	24.25±4.13	25.25±3.65	25.11±3.50
Waist circumference(cm)	82.16±12.25	81.87±11.78	90.55±9.64	89.68±9.88
Systolic blood pressure(mmHg)	123.28±19.14	123.53±19.53	131.97±18.46	131.51±16.37
Diastolic blood pressure(mmHg)	78.87±11.37	78.59±10.67	83.49±10.93	83.62±9.57
Adiponectin(μg/ml)	14.80(9.18-21.19)	14.86(9.76-21.68)	13.22(9.05-18.96)	13.60(8.35-20.92)
FPG(mmol/L)	4.90(4.50-5.30)	4.90(4.59-5.20)	7.48(6.42-9.45)	7.69(6.46-10.11)
PG2h(mmol/L)	5.94(4.80-6.71)	5.84(4.82-6.46)	14.48(11.98-17.41)	13.77(12.02-16.81)
FIN(mU/L)	7.51(4.86-11.78)	7.29(4.23-11.52)	11.83(7.13-19.06)	11.60(8.02-18.32)
IN2h(mU/L)	37.88(22.21-59.93)	35.47(19.79-62.59)	48.49(30.17-85.72)	47.10(28.41-76.70)
TC(mmol/L)	4.89±0.98	4.75±1.00	5.17±1.11	5.23±1.29
TG(mmol/L)	1.23(0.90-1.67)	1.28(0.88-1.80)	1.73(1.23-2.34)	1.78(1.23-2.51)
HDL-C(mmol/L)	1.34±0.29	1.29±0.33	1.12±0.27	1.14±0.40
LDL-C(mmol/L)	3.12±0.98	3.14±0.90	3.22±1.06	3.21±1.10
VA(cm^2)	86.84±57.29	76.19±44.51	112.56±43.03	108.44±40.57
SA(cm^2)	187.38±98.04	176.73±100.08	176.13±73.78	190.30±78.63
Stumvoll formula				
first phase IS	1256.27±475.09	1234.39±460.16	-373.60±1283.37	-449.76±1253.57
second phase IS	314.36±110.19	309.49±110.50	13.30±254.15	-1.69±233.16
Cederholm Index	3.07(2.62-3.62)	3.11(2.67-3.67)	1.34(1.15-1.56)	1.41(1.18-1.61)

Data were means ± SD for normal distribution variables, these variables were tested by *t*-test
Data were median value (25% Q1-75% Q3) for non-normal distribution variables, and these variables were Logarithmic transformed before *t*-test.
BMI, body mass index; FPG, fasting plasma glucose; PG2h, plasma glucose after 2 hours glucose load; FIN, fasting insulin; IN2h, insulin after 2 hours glucose load; TC, total cholesterol; TG, triglyceride; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; VA, visceral fat area; SA, subcutaneous fat area; IS, insulin secretion.

Clinical characteristics and DsbA-L genotypes

In addition to abnormal glucose metabolism, T2DM subjects had higher BMI after adjusted with sex and age, higher blood pressure, serum TC, TG concentrations, lower HDL-c concentrations, larger waist circumferences and visceral fat areas after adjusted with sex, age and BMI (data not shown) than those in normal subjects. The clinical characteristics of participants who carry *T/T* or *T/G* in both NGT and T2DM groups were compared with those of subjects carrying *G/G* and the results are summarized in Table 2. Our

Table 3. Insulin secretion according to the genotypes of the SNP-1308G/T (rs1917760) in NGT-NW subjects

	NGT BMI<24kg/m^2	
	T/T+T/G	*G/G*
Age(y)	55.49±17.08	52.24±17.16
Sex(M/F)	48/54	104/104
BMI(kg/m^2)	20.85±2.18	21.15±1.90
FIN(mU/L)	5.97(4.24-8.74)	5.74(3.38-8.63)
IN2h(mU/L)	33.23(19.13-52.73)	24.93(16.32-44.72)*
Stumvoll formula		
first phase IS	1173.70±313.03	1070.62±298.32*
second phase IS	293.76±77.46	271.52±79.19*
Cederholm index	3.35(2.68-3.85)	3.31(2.93-3.92)

Data were means ± SD for normal distribution variables, these variables were tested by *t*-test.
Data were median value (25% Q1 and 75% Q3) for non-normal distribution variables, and these variables were Logarithmic transformed before *t*-test.
* $P<0.05$
BMI, body mass index; FIN, fasting insulin; IN2h, insulin after 2 hours glucose load; IS, insulin secretion.

study revealed that there were no significantly genotype-related differences in age, sex distribution, blood pressure, total serum adiponectin concentrations, lipid profiles and body fat distribution between *T* carriers and non-*T* carriers in either T2DM group or NGT group. Significantly genotype-related differences were not found in HMW adiponectin concentrations in T2DM and NGT group.

It is interesting to notice that *T* carriers with higher BMI in NGT group (NGT-OW/OB) had more visceral fat areas than non-*T* carriers (116.96±5.40 cm^2 vs. 101.06±4.02 cm^2) after adjusted with sex, age and BMI (P=0.020). No genotype-related differences could be found in visceral fat areas in NGT-NW subjects.

Association of insulin sensitivity and insulin secretion with DsbA-L genotypes

There were no significant differences in plasma glucose, serum insulin concentrations, insulin secretion index (Stumvoll formula) and insulin sensitivity index (Cederholm index) according to this SNP in either NGT group or T2DM group (Table 2).

However, subjects with *T* allele had higher insulin concentrations after glucose loading than non-*T* carriers in NGT-NW group (P=0.036) after adjusted with sex, age and BMI (Table 3). Moreover, *T* allele carriers were found to have higher insulin secretion levels both in the first phase (P=0.007) and in second phase (P=0.031) than non-T carriers in the same group when calculated with the Stumvoll formula after adjustment with sex, age and BMI (Table 3, Figure 1). No genotype-related significant difference was found in Cederholm index in NGT-NW group. Since there were multiple tests in Table 2 and Table 3, we regulated the *P* values by *Bonferroni* method. However, all the *P*-values were not statistically significant after regulation.

In NGT-NW subjects, Pearson correlation analysis showed that when first phase insulin secretion was tested for simple linear correlations against genotypes, sex, age, BMI, and body fat distribution, significant correlations were found with the genotypes (r=-0.160; P=0.012) and age (r=-0.161; P=0.012). In second phase insulin secretion, a statistically significant correlation was also observed with genotypes (r=-0.134; P=0.036). Furthermore, multiple stepwise regression analysis revealed that SNP-1308G/T (rs1917760) genotypes were independently correlated with first phase insulin secretion according to Stumvoll formula in NGT-NW group (R^2=0.055; P=0.007). Similar results were obtained in second phase insulin secretion (R^2=0.029; P=0.041). Besides the polymorphism, HDL-c and LDL-c were also independently correlated with second phase insulin secretion.

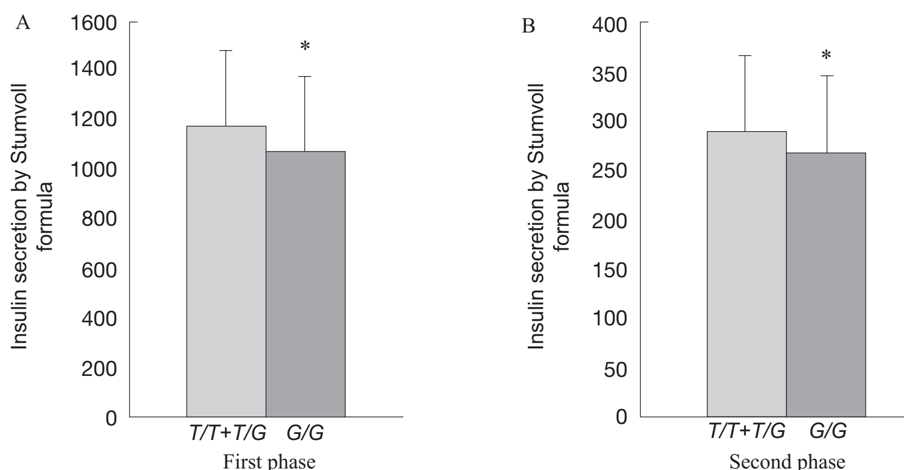

Fig. 1. Insulin secretion levels in NGT-NW subjects according to Stumvoll formula. The insulin secretion levels between *T/T+T/G* carriers (n=102) and *G/G* (n=208) carriers were compared by Student's t-test. A: First phase insulin secretion by Stumvoll formula; B: Second phase insulin secretion by Stumvoll formula. Data were presented as means ± SD. *$P<0.05$.

Discussion

In the present study, we investigated the association of polymorphism of DsbA-L gene with metabolic diseases, which has not been progressed before. According to the Hapmap and NCBI database, we selected the SNP-1308G/T (rs1917760) of the DsbA-L gene, which is in the 5' upstream of the DsbA-L gene.

First of all, genotype-related differences were found in insulin secretion levels after glucose loading in NGT-NW group in the present study. The SNP rs1917760 is in the 5' upstream of the DsbA-L gene. It is likely in the promoter region and might regulate the transcription of this gene. So we deduced that the change of the DsbA-L transcription might impact the insulin secretion level in pancreatic beta-cells. This outcome reveals a potential role of DsbA-L in pancreas. It has been reported that human DsbA-L mRNA was ubiquitously expressed in many tissues including pancreas [2]. Since DsbA family was a subfamily of the thioredoxin family and catalyzed disulfide bond formation during the folding of secreted proteins in bacteria, such as proinsulin [1, 2, 3], DsbA-L might also participate in the disulfide bond formation of insulin and impact insulin biosynthesis and secretion. Otherwise, it has also reported that DsbA-L mainly exists in peroxisomal and mitochondrial fractions [2]. Mitochondrial is important to the function or survival of pancreatic beta cells through the respiratory chain and oxidative stress [18, 19, 20, 21, 22]. Therefore, DsbA-L might play a role in pancreatic β cell survival in addition to regulating insulin assembling and secretion. The exact mechanisms underlying the function of DsbA-L in pancreatic beta cell need to be further investigated. The genotype-related differences in insulin secretion were mainly found in normal subjects with normal glucose tolerance and BMI<24kg/m^2. As other regulatory factors of insulin secretion should be normal in this group, the genotype of DsbA-L SNP may have an important role in regulating insulin secretion.

Multiple regression analysis revealed that the polymorphism of SNP-1308G/T (rs1917760) of DsbA-L gene was the variant independently associated with first phase insulin secretion in the NGT-NW subgroup. On the other hand, multiple variants had been identified to be independently associated with second phase insulin secretion, which include HDL-c, LDL-c, and polymorphism of DsbA-L gene. We speculate that subjects with higher HDL-c concentrations, lower LDL-c concentrations and *G/G* genotype secrete less insulin after glucose loading, which imply that this population might need less insulin and has better insulin sensitivity. These results were consistent with the previous reports that insulin resistance could cause low HDL-c concentrations and high LDL-c concentrations [23, 24]. The polymorphism of this SNP was independent variant associated with insulin secretion levels, but the R^2 values were relatively low, which suggested that this SNP had a minor effect on insulin secretion.

Another interesting result of the present study was that DsbA-L gene polymorphism was involved in body fat distribution. The subjects with *T* allele had larger visceral fat areas than those with non-*T* allele carriers in NGT-OW/OB subgroup. These results imply that this SNP might associate with abdominal obesity. Insulin resistance is one of the most important features of obesity; several studies had proved that abdominal obesity was more closely related to insulin resistance [25]. Hence, this SNP in DsbA-L gene might associate with insulin resistance.

We deduced that SNP-1308G/T (rs1917760) of DsbA-L gene might have an effect on insulin resistance through impacting visceral fat mass, but Cederholm index, which indicates insulin sensitivity, had no significant genotype-related differences in NGT-OW/OB subgroup. This implied that the SNP might only have an indirect effect on insulin resistance. The other potential reason could be that the sample size was relative small so the association between this SNP and insulin resistance was underestimated. Further investigations are needed to make a clear correlation between DsbA-L and insulin resistance.

Adiponectin is specifically and abundantly expressed in adipose tissue and directly sensitizes the body to insulin [26]. Serum adiponectin concentration is conversely related to obesity and type 2 diabetes [11]. Previous study showed that DsbA-L was highly expressed in fat tissue, and played a critical role in regulating adiponectin secretion and multimerization [4]. High molecular weight form (HMW) of adiponectin is the active form of the hormone and has a relevant role in enhancing insulin sensitivity and in protecting against diabetes [26]. However, this SNP of the DsbA-L gene did not associated with the total adiponectin and HMW adiponectin concentrations. The relationship of this SNP rs1917760 of the DsbA-L gene and the relationship of this protein itself with adiponectin need deeper investigations and functional studies are also necessary.

There was a limitation in the present study. After regulated by Bonferroni correction, all the *P* values of the continuous variables comparisons were not statistically significant. Thus, we could not exclude the possibility that our result is a false positive finding by chance. However, Bonferroni correction is too stringent given the fact that the traits we analyzed are tightly related. Further investigations in large population or in other races are necessary.

The molecular mechanisms by which DsbA-L acts as a modulator in insulin and adiponectin assembling/secretion and the relationship of DsbA-L with T2DM and obesity are largely unknown. Considering the complexity of the pathogenesis of T2DM and obesity, we speculated that the polymorphism of the DsbA-L gene might have some effects, but not causal one, on the development of T2DM and obesity.

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