Association of the $-243A>G$, $+61450C>A$ Polymorphisms of the Glutamate Decarboxylase 2 ($GAD2$) Gene with Obesity and Insulin Level in North Indian Population

Jai PRAKASH$^{1,2}$, Balraj MITTAL$^3$, Shally AWASTHI$^2$, *Neena SRIVASTAVA$^1$

1. Dept. Physiology, King George’s Medical University, Lucknow, India
2. Dept. of Pediatrics, King George’s Medical University, Lucknow, India
3. Dept. of Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India

*Corresponding Author: Email: neenasrivastavakgmc@gmail.com

(Received 18 Jan 2015; accepted 22 Oct 2015)

Abstract

Background: Obesity associated with type 2 diabetes, and hypertension increased mortality and morbidity. Glutamate decarboxylase 2 ($GAD2$) gene is associated with obesity and it regulate food intake and insulin level. We investigated the association of $GAD2$ gene $-243A>G$ (rs2236418) and $+61450C>A$ (rs992990) polymorphisms with obesity and related phenotypes.

Methods: Insulin, glucose and lipid levels were estimated using standard protocols. All subjects were genotyped (PCR-RFLP) method.

Results: The $-243A>G$ polymorphism of the $GAD2$ gene was significantly associated with higher risk of obesity ($P<0.05$).

Conclusion: $GAD2$ gene polymorphisms influence obesity and related phenotype in complex manner, probably by regulating the food intake, insulin and body weight.

Keywords: Obesity, Insulin, $GAD2$, Food intake, Polymorphism

Introduction

Obesity often begins early in life and is one of the most common non-communicable diseases and public health issues in the world at every stage of development. The magnitudes of these conditions include increased risk for cardiovascular diseases, diabetes, and many other diseases. The recent assessments specify that around 1.46 billion people globally are overweight and 502 million of them are obese (1). Obesity is strongly associated with significantly higher all-cause mortality during adulthood due to its association with various complex diseases (2).

Genome-wide scans for obesity genes have repetitively detected linkage with a locus on chromosome 10, has been linked to obesity (3,4). The first candidate gene for obesity identified through the genome wide approach was $GAD2$ (5). It encodes the glutamic acid decarboxylase enzyme GAD65 connected to obesity via the hypothalamic regulation of food intake. $GAD2$ regulates the formation of the $\gamma$-aminobutyric acid (GABA) from the glutamic acid. GABA functions together with neuropeptide Y in the paraventricular nucleus to stimulate food intake. In pancreatic islets, GABA modulates hormone secretion by pancreatic islets (6). Family-based genetic analyses showed association of obesity with other SNP, $+61450C>A$ (rs992990),

Available at: http://ijph.tums.ac.ir
which located in the GAD2 gene. −243A>G polymorphism (rs2236418) located in the promoter region of the GAD2 gene, has been associated with morbid obesity in various studies (5,7). The GAD2 gene polymorphisms were associated with childhood obesity, birth weight and binge eating (7). The G allele of −243A>G polymorphism was modestly associated with lower body mass index (BMI) and lower fasting glucose concentrations (8). Inconsistent results have been obtained regarding the association of GAD2 gene SNPs with obesity. In addition, other studies involving European and American population showed no association with obesity (3, 9, 10).

Now, obesity and obesity associated phenotypes becomes very common and both environmental as well as genetic factors significantly contribute toward susceptibility of obesity. As the association studies are influenced by populations and ethnicities, we ascertained the role of these SNPs in genetic susceptibility to obesity and obesity associated phenotypes in our north Indian population.

**Materials and Methods**

**Subjects**

The population studied included 642 apparently healthy individuals (347 men, 295 women) ranging in age from 19 to 60 yr. The samples were collected randomly from the outpatients department of King George’s Medical University, Lucknow and from the general population of Lucknow (North India). 309 obese (BMI > 30 k.g/m²) and 333 non obese (BMI ≤ 30 k.g/m²) individual were participated in the study, selected based on the strict inclusion criteria. In all subjects’ body height, body weight, waist circumferences and hip circumferences were measured for calculation of BMI and waist to hip ratio (WHR). The Subjects were free from serious and/or chronic illnesses, especially diabetes mellitus, coronary artery disease and pregnant women were excluded. All individuals were of north Indian origin and the population was homogeneous with regard to ethnic background (11). Subjects were classified as type 2 diabetes patients if they were on hypoglycemic medications or when their fasting glucose concentrations exceeded 126 mg/dL. Prior informed written consent was taken from each participant and the identity of all participants was kept confidential. All protocols and the study were carried out with the approval of local ethics committee (IRB number- XXXIV ECM/P6) at King George’s Medical University Lucknow.

At baseline all study participants were subjected to a thorough screening program that included assessment of a detailed personal and family history, physical examination, determination of anthropometric indices and measurement of various biochemical parameters were done.

**Estimation of body fat composition**

The Body fat analyzer (Bioelectrical impedance was obtained using a device, Tanita–TBF–310, Japan) was used for assessing the BMI, %body fat, fat mass (FM), fat free mass (FFM) and total body water (TBW).

**Laboratory measurements**

Venous blood was collected after an overnight fast, and was centrifuged within 2 h after collection. Plasma samples were immediately separated and stored in aliquots at −80°C until they were analyzed. Commercial enzymatic tests were used for determining fasting glucose, lipid profile while low density lipoprotein (LDL) cholesterol was calculated by the formula of Friedewald. Fasting insulin level was determined by enzyme-linked immunosorbent assay RIA (Linco Research). The degree of insulin sensitivity/resistance was calculated according to the homeostasis model assessment (HOMA) which is a good index for assessing insulin sensitivity/resistance. A description of each method was given by us elsewhere (12). GAD2 SNPs were genotyped by PCR-based restriction fragment length polymorphism analysis or by tetra amplification refractory mutation system PCR (9).

**Quality control**

Quality control and assessment was done at every step of the study. The amount of isolated DNA was of good quality (Absorbance 260 nm/280 nm
ratio >1.75). One sample with known genotype and a reagent blank were included after every 20 samples in the PCR. A 50 base-pair marker was included during electrophoresis. Twenty percent of samples from patients and controls, including samples of each genotype were re-genotyped by other laboratory personnel. No discrepancy was found after sequencing the randomly selected 5% samples.

**Statistical analysis**

Statistical analyses were performed using SPSS 15 (Chicago, IL) statistical tools. The independent segregation of alleles was tested for the Hardy-Weinberg equilibrium (HWE), comparing the observed genotype frequencies with those expected ($X^2$ test). Genotype and allele distribution was compared between obese and non-obese subjects using $X^2$ test. Haplotype analysis was done by SNP analyzer version 1.2 by expectation-maximization algorithm (13). Most frequent homozygous genotype in the control group was used as reference for association analysis. Statistical significant was determined at $P<0.05$ for all tests. The hypothesis of different genetic models (Additive model, Co-dominant model, Dominant model, Over dominant model, and Multiplicative model) used in the study is described in Table 1. The differences among the three groups were assessed by one-way ANOVA for continuous variables and variables are given as the mean ± SD.

| SN | Type of models     | Hypothesis                                      |
|----|--------------------|-------------------------------------------------|
| 1  | Additive model     | r-Fold increased risk for genotype with one risk (minor) allele and 2-fold increased risk for homozoygous variant or with two risk (minor) allele |
| 2  | Co-dominant model  | Risk genotype with two minor allele and wild genotype with two major alleles |
| 3  | Dominant model     | Risk genotypes with at least one minor allele and wild genotype with two major alleles |
| 4  | Recessive model    | Risk genotype with two minor allele and wild genotype with at least one major allele |
| 5  | Over dominant model| Risk genotypes with two minor allele or two alleles and wild genotype with one minor allele and one major allele |
| 6  | Multiplicative model| r-Fold increased risk for hetero genotype and r$^2$ increased risk for homozygous minor allele |

**Results**

Clinical characteristics of the obese (BMI≥30) and non-obese (BMI<30) participants of the present study are shown in Table 2. Systolic blood pressure, diastolic blood pressure, FM (fat mass), % body fat, WHR, insulin, HOMA-IR, and lipid profile were significantly different between obese and non-obese subjects.

**Genetic association with obesity**

The GAD2 gene polymorphisms (rs2236418 and rs992990) were genotyped in all the subjects. There was no significant deviation from the Hardy-Weinberg equilibrium among the subjects with respect to both the SNPs ($P<0.05$). The distributions of allele and genotype and haplotype frequencies along with their odds ratios among obese and non-obese subjects are presented in Table 3. Overall -243 A>G polymorphism shows significantly higher minor allele and genotype frequencies in obese cases, when compared with non-obese subjects (odds ratio (OR), 4.01; 95% confidence interval (CI), 1.91-8.42; $P=0.001$). The genotype and allele frequencies of SNP +61450 C>A was not different in obese subjects compared with non-obese subjects and showed no association with obesity.

A positive significant association has also been observed in the additive, dominant and recessive model for SNP (rs2236418: OR 1.52; $P=0.016$; OR 1.75; $P=0.001$, OR 3.47; $P=0.001$), respectively, while no significant association has been seen in over dominant genetic model. However, no association has been seen in any genetic model for the SNP rs992990. The haplotype consisting of wild type alleles of two-studied variable (A-C) were represented more often in the non-obese group (61.0%) than in the obese group (49.8%). The frequency of combined haplotypes CG+AG was observed significantly higher in obese than non-obese (OR 1.94, $P=0.001$), Table 4.
Table 2: Demographic characteristic of the study subjects

| Variable                | Non-obese (n=333) | Obese (n=309) | P-value |
|------------------------|-------------------|---------------|---------|
| Waist-to-hip ratio     | 0.95 (0.08)       | 0.96±0.10     | 0.025   |
| SBP (mm Hg)            | 120.51±12.00      | 128.40±15.20  | <0.001  |
| DBP (mm Hg)            | 81.00±8.00        | 86.23±8.05    | 0.027   |
| Adiposity              |                   |               |         |
| Weight (kg)            | 69.00±6.00        | 78.40±7.42    | 0.032   |
| % body fat             | 27.86±6.12        | 37.28±6.16    | <0.001  |
| FM (kg)                | 20.60±8.16        | 30.60±8.33    | <0.001  |
| Insulin sensitivity    |                   |               |         |
| Fasting glucose (mg/dl)| 109.22±5.93       | 109.63±8.61   | NS      |
| Fasting insulin (µU/ml)| 10.27±3.01        | 14.99±4.73    | <0.001  |
| Homa Index             | 2.83±1.83         | 4.15±2.86     | <0.001  |
| Lipid profile          |                   |               |         |
| T Cholesterol (mg/dl)  | 161.71±34.70      | 213.53±25.71  | 0.003   |
| HDL Cholesterol (mg/dl)| 46.30±10.16       | 42.82±7.13    | <0.001  |
| VLDL Cholesterol (mg/dl)| 21.42±3.91      | 26.05±5.77    | 0.008   |
| Triglyceride (mg/dl)   | 107.12±12.57      | 130.27±21.88  | 0.008   |
| LDL Cholesterol (mg/dl)| 99.68±27.07       | 151.28±22.44  | <0.001  |

Values are expressed in Mean±SD; The bold values represent statistically significant values less than 0.05.

Table 3: Distribution of GAD2 gene among non-obese (n=333) and obese subjects (n=309)

| Genotype                | Non-obese n (%) | Obese n (%) | OR (95% CI) | P-value |
|------------------------|-----------------|-------------|-------------|---------|
| GAD2 (rs2236418;−243 A>G) |                 |             |             |         |
| AA                     | 227 (68.2)      | 170 (55.0)  | 1           |         |
| AG (additive model)    | 96 (28.8)       | 109 (35.3)  | 1.52 (1.08-2.13) | 0.016  |
| GG (co-dominant model) | 10 (3.0)        | 30 (9.7)    | 4.01 (1.91-8.42) | <0.001 |
| AG+GG (dominant model) | 106 (31.8)      | 139 (45.0)  | 1.75 (1.27-2.42) | <0.001 |
| GG versus AG+AA (recessive model) | 323 (97.0) | 299 (90.3)  | 3.47 (1.67-7.23) | 0.001  |
| AA+GG versus AG (over dominant model) | 237 (71.2) | 200 (64.7)  | 0.74 (0.53-1.04) | 0.080  |
| Allele frequency       |                 |             |             |         |
| A (multiplicative model)| 550 (82.6)     | 449 (72.7)  | 0.56 (0.43-0.73) | <0.001 |
| G (multiplicative model)| 116 (17.4)     | 169 (27.3)  | 1.79 (1.37-2.33) | <0.001 |
| GAD2 (rs992990;+61450 C>A) |             |             |             |         |
| CC                     | 204 (61.3)      | 190 (61.5)  | 1           |         |
| CA (additive model)    | 77 (23.1)       | 75 (24.3)   | 1.05 (0.72-1.52) | 0.815  |
| AA (co-dominant model) | 52 (15.6)       | 44 (14.2)   | 0.91 (0.58-1.42) | 0.674  |
| CA+AA (dominant model) | 129 (38.7)      | 119 (38.5)  | 0.990 (0.721-1.361) | 0.953  |
| AA versus CA+CC (recessive model) | 281 (84.4) | 265 (85.8)  | 0.897 (0.581-1.386) | 0.625  |
| CC+AA versus CA (over dominant model) | 256 (76.9) | 234 (75.7)  | 1.066 (0.760-1.534) | 0.732  |
| Allele frequency       |                 |             |             |         |
| C (multiplicative model)| 485 (72.8)     | 455 (73.6)  | 1.042 (0.814-1.334) | 0.726  |
| A (multiplicative model)| 181 (27.2)     | 163 (26.4)  | 0.960 (0.750-0.1229) | 0.746  |

The bold values represent statistically significant values < 0.05.

Table 4: Haplotype frequency of gene variants and its association with risk of obesity

| Haplotype | Non-obese n (%) | Obese n (%) | OR (95% CI) | P-value |
|-----------|-----------------|-------------|-------------|---------|
| CA        | 203 (61.0%)     | 154 (49.8%) | 1           | Reference |
| AA        | 73 (21.9%)      | 71 (23.0%)  | 1.28 (0.87-3.189) | 0.210  |
| CG+AG     | 57 (17.1%)      | 84 (27.2%)  | 1.94 (1.31-2.89) | 0.001  |

The bold values represent statistically significant values < 0.05; The bold values represent statistically significant values less than 0.05.

Available at: [http://ijph.tums.ac.ir](http://ijph.tums.ac.ir)
Association of different metabolic parameters with homozygous variant genotype

GAD-2 rs2236418 showed association with different obesity associated phenotypes like weight, percentage body fat, FM, fasting blood glucose, and insulin (Table 5). The strongest association was found with weight and insulin. The GAD-2 rs992990 polymorphism also showed association with weight, percentage body fat, FM and insulin (Table 6).

![Table 5: Association of GAD2 -243 A>G, (rs2236418) genotypes with obesity related phenotypes](http://ijph.tums.ac.ir)

| Phenotype       | AA (n=397)       | AG (n=205)       | GG (n=40)       | P-value |
|-----------------|------------------|------------------|-----------------|---------|
| Waist-to-hip ratio | 0.95 (0.95-0.96) | 0.96 (0.95-0.98) | 0.98 (0.96-0.99) | 0.855   |
| SBP (mm Hg)     | 123.36 (122.21-125.15) | 125.03 (123.32-127.49) | 125.92 (122.69-127.56) | 0.159   |
| DBP (mm Hg)     | 82.83 (82.21-83.88) | 83.07 (81.90-84.98) | 84.49 (82.90-85.63) | 0.09    |
| Weight (kg)     | 71.49 (70.55-72.39) | 76.32 (75.42-77.65) | 79.31 (78.53-80.87) | 0.001   |
| % body fat      | 31.35 (30.87-32.39) | 33.28 (32.65-34.99) | 33.31 (31.57-34.97) | <0.001  |
| FM (kg)         | 24.15 (23.44-25.30) | 26.56 (24.88-29.11) | 27.12 (25.59-28.64) | 0.018   |
| Insulin sensitivity | 106.54 (105.72-107.87) | 114.10 (112.83-115.34) | 114.68 (113.40-115.65) | 0.039   |
| Fasting insulin (µU/ml) | 22.22 (19.70-24.53) | 17.19 (16.80-18.51) | 8.19 (7.82-8.66) | <0.001  |

Data represents mean and its 95% confidence interval from ANOVA; *P values were derived from multiple linear regression analysis using the best genetic model; The bold values represent statistically significant values less than 0.05.

![Table 6: Association of GAD2 +61450 C>A, (rs992990) genotypes with obesity related phenotypes](http://ijph.tums.ac.ir)

| Phenotype       | CC (n=394)       | CA (n=152)       | AA (n=96)       | P-value |
|-----------------|------------------|------------------|-----------------|---------|
| WHR             | 0.95 (0.95-0.97) | 0.96 (0.95-0.98) | 0.97 (0.94-0.99) | 0.905   |
| SBP (mm Hg)     | 123.63 (122.33-124.93) | 125.38 (123.47-127.30) | 125.45 (118.58-132.32) | 0.216   |
| DBP (mm Hg)     | 82.87 (82.08-83.67) | 83.88 (82.69-85.08) | 86.05 (83.33-88.77) | 0.004   |
| Adiposity       | 72.75 (71.33-74.17) | 74.39 (69.90-78.87) | 75.68 (73.65-77.70) | 0.001   |
| Weight (kg)     | 31.19 (30.49-31.90) | 33.85 (32.72-34.97) | 36.85 (34.14-39.56) | <0.001  |
| % body fat      | 24.05 (23.19-24.91) | 27.27 (25.84-28.70) | 29.41 (25.72-33.09) | 0.015   |
| FM (kg)         | 108.71 (106.43-110.98) | 109.58 (107.87-111.28) | 111.58 (104.88-118.29) | 0.019   |
| Insulin sensitivity | 12.85 (12.04-13.65) | 12.41 (11.19-13.64) | 10.21 (7.88-12.54) | <0.001  |

Data represents mean and its 95% confidence interval from ANOVA; *P values were derived from multiple linear regression analysis using the best genetic model; The bold values represent statistically significant values less than 0.05.

Discussion

In the present study, we found association of SNPs in GAD2 (genotype, allele and haplotype) with the predisposition to human obesity and its association with insulin secretion. We observed significant association of GAD-2 -243 A>G polymorphism at genotype and allele level with risk of obesity while GAD-2 +61450 C>A polymorphism do not associated with obesity. The variant genotypes of -243 A>G and +61450 C>A polymorphism also associated with weight, % body fat and fat mass in obese and non-obese subjects both (Table 5 & 6). Haplotypes were estimated from -243 A>G and +61450 C>A for each subject and tested for differences between the obese and non-obese subjects by logistic regression. There were two common haplotypes: CA, and AA, with the remaining haplotypes being combined into a single haplotype (CG+AG). The
combined haplotype showed significant association with obesity in the present study (P value = 0.001, Table 4). Table 7 shows the list of various studies on the GAD-2 polymorphisms related to obesity. The (−243) A>G polymorphism of the GAD2 gene showed a strong association with obesity (5), while other studies report the contradictory results showing no association with obesity (3,9,10). The contribution of GAD2 promoter SNP -243 A<G to the genetic risk for childhood obesity and with lower BMI, fasting glucose concentrations were observed (8). In addition, investigations into the functional consequences of the (−243) A→G SNP in the promoter region of the GAD2 gene have reported contradictory results (5,9).

+61450 C>A polymorphism was not associated with obesity, so we did (9). The GAD-2 +61450 C>A polymorphism did not show association with obesity in Utah population (10). However, a family-based genetic study, reported significant association of +61450 C>A polymorphism with obesity (5).

GAD2 encodes the 65-kDa isoform of the enzyme glutamate decarboxylase, which enhance catalyses of the production of c-aminobutyric acid (GABA) from glutamic acid that interact with neuropeptide Y in neurons of the hypothalamic arcuate nucleus to stimulate food intake (14). These acute neuropeptide Y neurons also slow down the similarities and opposite effects of neighbouring pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript neurons via GABA-ergic collateral inputs (15). Food intake suppressed after the administration of the antisense GAD-65 and GAD-67 oligonucleotides by inhibiting the GABA synthesis in the ventromedial hypothalamus (16).

| GAD2 -243 A>G polymorphism (rs2236418) | Reference |
|---------------------------------------|-----------|
| Association with obesity              | (5)       |
| Association with obesity              | (7)       |
| No association with obesity           | (9)       |
| No association with obesity           | (10)      |
| No association with obesity           | (8)       |
| Association with resting energy expenditure | (23)     |
| Association with obesity              | Present study |
| GAD2 +61450 C>A polymorphism (rs992990) | Present study |
| Association with obesity              | (5)       |
| No association with obesity           | (9)       |
| No association with obesity           | (10)      |
| No association with obesity           | (24)      |
| Susceptibility to hunger& weight gain | (25)      |
| No association with obesity           | Present study |

In addition, GAD2 gene polymorphisms showed significant association with the insulin level in obese and non-obese subjects. GABA pool in the hypothalamus may enhance by an increased activity of the GAD2 gene and improved orexigenic effect of GABA resulting in changed feeding behaviour. Denbow et al. (17) observed the effect of varying doses of muscimol (GABA agonist) on food intake. GAD2 SNP-related increase of the GABA pool in pancreatic islets also contributes in food intake (18). Heritability of obesity with improved body weight and fat deposition showed in transgenic mice with increased expression of GABA trans-
porter (19). The GAD2 gene is highly expressed in pancreatic b cells. GABA-containing micro vesicles are released by Ca2þ-dependent exocytosis and may have an autocrine/paracrine function within the islet (20). GABA released from the b cell inhibits insulin exocytosis by activation of GABA-B receptors (G protein-coupled receptors) (21). The significant association between GAD2 promoter SNP (SNP+83897 T>A) and lower level of insulin in non-obese subjects were observed while no association was reported in obese subjects (5). The association of variant allele of -243 A<G SNP with 6-fold increased GAD2 gene promoter activity (5). Increased GABA level in the central nervous system encouraged enhanced food intake and obesity (17, 19). In the present study we found the association between variant genotype of -243A>G and +61450 C>A and lower fasting insulin levels and HOMA-IR indexes which suggest the deleterious effect of GAD2 SNPs on insulin secretion. Insulin secretion is strongly inhibited by GABA, released from b cells. An increase of the GABA pool in pancreatic b cells mediated by GAD65 higher enzymatic activity may be responsible for above explained association (22).

-243 A>G SNP and +61450 C>A SNP of the GAD2 gene in linkage disequilibrium, variant allele, genotype of -243 A>G SNP and combined haplotypes of -243 A>G SNP and +61450 C>A SNP (CG+AG) showed the strongest association with obesity. Functional studies were performed to analyse its effects on transcription and nuclear protein binding. The (-243A>G) SNP increased transcriptional activity and promoter activity by which GABA production stimulated, may possibly have effect on feeding and insulin (5). Lack of data, in particular, lifestyle factors such as education, occupation, per capita income, population social background, and daily activity patterns, in the present analysis are an important limitation of this study. An additional limitation of the study is multiple comparisons; however, the majority of literature to date would indicate that the GAD2 gene variant is associated with measures of insulin secretion. Other influencing factors small sample size of the study which may reduce the capability to identify certain associations. Lastly, as our study nature is cross-sectional, so we are limited to identifying associations only and longitudinal studies required to identify and understand more clearly the role of GAD2 gene. However, this study also has a number of significant advantages: the present sample was collected from an ethnically and socioeconomically homogeneous population, with phenotype measurements not modified by medication. The study's participants were evaluated simultaneously for all the studied variables.

Conclusion

We reported that -243 A>G SNP, and the combined haplotypes of GAD2 gene significantly associated with obesity in north Indian population, while +61450 C>A SNP did not show association. However, an association between variant genotype of -243 A>G, +61450 C>A SNP with body weight and insulin level were observed. It is evident that GAD2 variants have a detectable influence on obesity and obesity related phenotype like insulin secretion in a diverse range of populations. Future studies of the association of these SNPs with obesity and obesity related abnormalities may more rigorously prove the suggested statistical association.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgments

The authors acknowledge the Indian Council of Medical Research, New Delhi, for the financial support to carry out this research work (Project number - 45/14/2008-HUM-BMS). No compet-
ing financial interests exist. The authors declare that there is no conflict of interests.

References

1. Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, Singh GM, Gutierrez HR, Lu Y, Bahalim AN, Farzadfar F, Riley LM, Ezzati M (2011). Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Body Mass Index). National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9·1 million participants. Lancet, 12:557-567.

2. Flegal K, Kit B, Orpana H, Graubard B (2013). Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and metaanalysis. JAMA, 309:71-82.

3. Groves CJ, Zeggini E, Walker M, Hitman GA, Levy JC, O’Rahilly S, Hattersley AT, McCarthy MI, Wiltshire S (2006). Significant linkage of BMI to chromosome 1p10 in the U.K. population and evaluation of GAD2 as a positional candidate. Diabetes, 55:1884-89.

4. Karlsen AE, Hapogian WA, Grubin CE, Dube S, Distecho CM, Adler DA, Bärmeier H, Mathewes S, Grant FJ, Foster D (1991). Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10. Proc Natl Acad Sci U S A, 1:8337-41.

5. Boutin P, Dina C, Vasseur F, Dubois S, Corset I, Séron K, Bekris L, Cabellon J, Neve B, Vasseur-Delannoy V, Chikri M, Charles MA, Clement K, Lenmark A, Froguel P (2003). GAD2 on chromosome 10p12 is a candidate gene for human obesity. PLoS Biol, 1:E68.

6. Wang C, Mao R, Van de Casteele M, Pipeleers D, Ling Z (2007). Glucagon-like peptide-1 stimulates GABA formation by pancreatic beta-cells at the level of glutamate decarboxylase. Am J Physiol Endocrinol Metab, 292:E1201-6.

7. Meyre D, Boutin P, Tounian A, Deweirder M, Aout M, Jouret B, Heude B, Weill J, Tauber M, Tounian P, Froguel P (2005). Is glutamate decarboxylase 2 (GAD2) a genetic link between low birth weight and subsequent development of obesity in children? J Clin Endocrinol Metab, 90:2384-90.

8. Boesgaard TW, Castella SI, Andersen G, Albrectsen A, Sparso T, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O (2007). A -243A->G polymorphism upstream of the gene encoding GAD65 associates with lower levels of body mass index and glycaemia in a population-based sample of 5857 middle-aged White subjects. Diabet Med, 24:702-6.

9. Swarbrick MM, Waldenmaier B, Pennacchio LA, Lind DL, Cavazos MM, Geller F, Merriman R, Ustaszewska A, Malloy M, Schug A, Hsueh WC, Rief W, Maurais-Jarvis F, Pullinger CR, Kane JP, Dent R, McPherson R, Kwok PY, Hinney A, Hebebrand J, Väisäe C (2005). Lack of support for the association between GAD2 polymorphisms and severe human obesity. PLoS Biol, 3:e315.

10. Hunt SC, Xin Y, Wu LL, Hopkins PN, Adams TD (2006). Lack of association of glutamate decarboxylase 2 gene polymorphisms with severe obesity in Utah. Obesity, 14:650-55.

11. Prakash J, Srivastava N, Awasthi S, Agarwal C, Natu S, Rajpal N, Mittal B (2012). Association of PPAR-γ gene polymorphisms with obesity and obesity-associated phenotypes in North Indian population. Am J Hum Biol, 24:454-59.

12. Prakash J, Mittal B, Awasthi S, Agarwal CG, Srivastava N (2013). K121Q ENPP1/PC-1 gene polymorphism is associated with insulin resistance in a North Indian population. J Genet, 92:571-76.

13. Yoo J, Seo B, Kim Y (2005). SNP Analyzer: a web-based integrated workbench for single-nucleotide polymorphism analysis. Nucleic Acids Res, 33:W483-88.

14. Ovesjo ML, Gamstedt M, Collin M, Meister B (2001). GABAergic nature of hypothalamic leptin target neurones in the ventromedial arcuate nucleus. J Neuroendocrinol, 13:505-16.

15. Cowley MA, Smart JL, Rubinstein M, Cerdin MG, Diano S, Horvath TL, Cone RD, Low MJ (2001). Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature, 24:480-84.

16. Bannai M, Ichikawa M, Nishihara M, Takahashi M (1998). Effect of injection of antisense oligodeoxynucleotides of GAD isozymes into rat ventromedial hypothalamus on food

Available at: http://ijph.tums.ac.ir
intake and locomotor activity. *Brain Res*, 784:305-15.

17. Denbow DM (1991). Induction of food intake by a GABAergic mechanism in the turkey. *Physiol Behav*, 49:485-88.

18. Niswender KD, Morrison CD, Clegg DJ, Olson R, Baskin DG, Myers MG Jr, Seeley RJ, Schwartz MW (2003). Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. *Diabetes*, 52:227-31.

19. Ma YH, Hu JH, Zhou XG, Zeng RW, Mei ZT, Fei J, Guo LH (2000). Transgenic mice overexpressing gamma-aminobutyric acid transporter subtype I develop obesity. *Cell Res*, 10:303-310.

20. Gu XH, Kurose T, Kato S, Masuda K, Ishida H, Seino Y. (1993). Suppressive effect of GABA on insulin secretion from the pancreatic beta-cells in the rat. *Life Sci*, 52:687-94.

21. Rorsman P, Berggren PO, Bokvist K, Ericson H, Möhler H, Ostenson CG, Smith PA (1989). Glucose-inhibition of glucagon secretion involves activation of GABAA-receptor chloride channels. *Nature*, 21:233-36.

22. Shi Y, Kanaani J, Menard-Rose V, Ma YH, Chang PY, Hanahan D, Tobin A, Grodsky G, Baekkeskov S (2000). Increased expression of GAD65 and GABA in pancreatic beta-cells impairs first-phase insulin secretion. *Am J Physiol Endocrinol Metab*, 279:E684-94.

23. Goossens GH, Petersen L, Blaak EE, Hul G, Arner P, Astrup A, Froguel P, Patel K, Pedersen O, Polak J, Oppert JM, Martinez JA, Sørensen TI, Saris WH (2009). Several obesity- and nutrient-related gene polymorphisms but not FTO and UCP variants modulate postabsorptive resting energy expenditure and fat-induced thermogenesis in obese individuals: the NUGENOB study. *Int J Obes (Lond)*, 33:669-79.

24. Chen KC, Lin YC, Chao WC, Chung HK, Chi SS, Liu WS, Wu WT (2012). Association of genetic polymorphisms of glutamate decarboxylase 2 and the dopamine D2 receptor with obesity in Taiwanese subjects. *Ann Saudi Med*, 32:121-26.

25. Choquette AC, Lemieux S, Tremblay A, Drapeau V, Bouchard C, Vohl MC, Pérusse L (2009). GAD2 gene sequence variations are associated with eating behaviors and weight gain in women from the Quebec family study. *Physiol Behav*, 19:505-10.

### List of abbreviations (in alphabetical order)

| #  | Abbreviation | Explanation                                                                 | Units         |
|----|--------------|-----------------------------------------------------------------------------|---------------|
| 1  | ANOVA        | Analysis of covariance                                                      |               |
| 2  | BMI          | Body mass index                                                             | kg/m²         |
| 3  | DBP          | Diastolic blood pressure                                                    | mm Hg         |
| 4  | FM           | Fat mass                                                                    | kg/m²         |
| 5  | GABA         | γ-aminobutyric acid                                                         |               |
| 6  | HDL-cholesterol | High density lipoprotein – cholesterol                                   | mg/dl         |
| 7  | HOMA         | Homeostasis model assessment                                                |               |
| 8  | HOMA-IR Index | Homeostasis model assessment of insulin resistance index                    |               |
| 9  | LDL cholesterol | Low density lipoprotein – cholesterol                                      | mg/dl         |
| 10 | PCR-RFLP     | Polymerase chain reaction restriction fragment length polymorphism          |               |
| 11 | SBP          | Systolic blood pressure                                                    | mm Hg         |
| 12 | T cholesterol | Total – cholesterol                                                        | mg/dl         |
| 13 | VLDL cholesterol | Very low density lipoprotein – cholesterol                                | mg/dl         |
| 14 | WHR          | Waist to hip ratio                                                          |               |
| 15 | GAD2         | Glutamate decarboxylase 2                                                   |               |

**Human Gene**