Obesity, an abnormal excessive storage of body fat, has contributed to the gradually increasing list of health complications, such as heart disease, type 2 diabetes, breathing difficulties during sleep, hypertension, dyslipidemia, heart failure, stroke, cancers, and osteoarthritis. The definition of obesity is based on body mass index (BMI), an index of weight-for-height that is associated with the content of body fat. It is well known that obesity is most commonly caused by a combination of excessive dietary calories, lack of physical activity, and genetic susceptibility. The causes of obesity are debated and studied, but previous studies have suggested that some reasons behind morbid obesity may include a genetic predisposition, endocrine disorders, medications, or psychiatric illness.

It has been demonstrated that the glutamate decarboxylase 2 gene (GAD2) on the locus of chromosome 10p12 is the candidate gene concerned for craving behavior and weight gain, and is associated with severe human obesity. The glutamate decarboxylase 2 (GAD2) plays a role in catalyzing the production of the γ-aminobutyric acid (GABA) neurotransmitter in neurochemical pathway, and regulates the release of dopamine (DA) in the nucleus accumbens site. DA is known as a pleasure or antistress molecule, and it is involved in the events of the brain reward cascade.
The dopamine D2 receptor gene (DRD2), located on chromosome 11q23, encodes the D2 subtype of the DA receptor, to maintain normal craving behaviors. More recent data has indicated that a lack of D2 receptors causes subjects to have a high risk for multiple addictive, impulsive, and compulsive behaviors, such as alcoholism, glucose binging, sex addiction, and antisocial behaviors. Consistently, an amino acid enkephalinase known as synaptamine potentially induces DA release and stimulates the proliferation of D2 receptors and promotes the attenuation of abnormal behaviors. DA deficiency is usually due to an association with the DRD2 gene A1 allele and other gene variations involved in the reward cascade. This reduces DA release and/or receptivity and has been described as the reward deficiency syndrome (RDS).

Pharmacological studies have described that cannabinoid and opioid receptor antagonists could potentially attenuate alcohol addiction and help to control alcohol intake and reduce the motivation to consume alcohol. Recently, a novel therapeutic approach was proposed to reduce many harmful craving behaviors by using immunologically compatible substances, through the help of a genetic positioning system map. Individuals with genetically compatible substances, through the help of an amino acid enkephalinase, presented a high risk for multiple addictive, impulsive, and compulsive behaviors. Consistently, an amino acid enkephalinase known as synaptamine potentially induces DA release and stimulates the proliferation of D2 receptors and promotes the attenuation of abnormal behaviors. DA deficiency is usually due to an association with the DRD2 gene A1 allele and other gene variations involved in the reward cascade. This reduces DA release and/or receptivity and has been described as the reward deficiency syndrome (RDS).

DNA extraction

Total genomic DNA was extracted with the DNeasy Kit (Qiagen, USA) according to the manufacturer’s instructions. The blood was digested with 0.5 mg/mL proteinase K in 400 µL cell- lysis solution for 24 hours at 55°C until the blood was completely lysed. After adding 200 µL absolute ethanol to the lysed sample, the mixture was transferred into the DNeasy mini column and centrifuged for 1 minute at 8000 revolutions per minute (rpm). The DNeasy mini column was washed with 500 µL washing buffer and centrifuged for 1 minute at 8000 rpm. Finally, the DNA was eluted in a clean 1.5-mL microcentrifuge tube. The amount of DNA was measured spectrophotometrically using a spectrophotometer (GeneQuant, GE Healthcare Bio-Sciences AB, Sweden) and stored at –20°C until DNA extraction.

Gene polymorphisms

The T/A substitution at position +83897 in the DRD2 gene was assessed by PCR amplification and the products were submitted to digest. The sequences of PCR primers were 5’-GTG GCA GGC AGC TGA TAG TC-3’ (sense) and 5’-CAC CTG TGG GAC AGA CCA TA-3’ (antisense) with an expected PCR product size of 242 bp. Amplification was performed by using a Perkin-Elmer 9700 thermal cycler (Applied Biosystems, Foster City, CA) and polypropylene PCR plates no. 170651 (Biozym, Landgraaf, The Netherlands). The amplification conditions consisted of 94°C for 3 minutes, followed by 45 cycles of 94°C for 1 minute, 56°C for 1 minute, and 72°C for 40 seconds. The reaction was terminated by a final elongation at 72°C for 7 minutes. The products were digested with 5 U/µL of AluI at 37°C for 2 hours and formed 146- and 96-bp DNA fragments.
products for allele T and an intact fragment of 242-bp DNA products for allele A. The digested products were separated on a 2.5% agarose gel stained with ethidium bromide (0.5 µg/mL), and genotypes were determined by analyzing different bands. The C/A substitution at position +61450 in the \textit{GAD2} gene was genotyped by tetra-primer amplification refractory mutation system (ARMS)-PCR by using 2 primer pairs to amplify, respectively. The primers were as follows: GAD-61450-FiC 5’-ATT CTT ACT GAC AAA GCT GAG TTT ACC C-3’ and GAD-61450-Ro 5’-TAT TTA GGT GAA GTG CTT AGA ACT GTG C-3’, the 199 bp for detecting the C allele; and GAD-61450-RiA 5’-TCA TGT TCT ATG GCT AGA TGT CTA ATC CT-3’ and GAD-61450-Fo 5’-GGC AGC TTC TCT AAA AAG ACA AAT A-3’, the 151 bp for detecting the A allele. The S311C variant in \textit{DRD2} was genotyped by amplification of the corresponding DNA fragment according to the PCR method. The 294 bp PCR fragments were digested with Sau961 restriction enzyme (New England Biolabs, United Kingdom). The Ser311 allele has 4 bands of 126, 92, 53, and 23 bp, whereas the Cys311 allele has 3 bands of 149, 92, and 53 bp. Genotyping was checked by two readers who were blinded to the clinical data.

\textbf{Statistical analysis}

Genotype and allele frequencies were compared by analysis of variance (ANOVA) and the $\chi^2$ test for small sample size. The P value, odds ratios, and 95\% confidence interval were calculated. A $P$ value of less than .05 was significant for all analyses.

\textbf{RESULTS}

Women and men comprised approximately equal proportions of the case and control groups (\textit{Table 1}). Since men and women differ in adiposity, women are more liable to being obese than men. The frequencies of \textit{GAD2} (+83897 T/A) TT, TA, and AA genotypes were 31.8\%, 46.3\%, and 21.9\% in cases and 14.9\%, 52.9\%, and 26.2\% in controls, respectively (\textit{Table 2}). The distribution of \textit{GAD2} (+83897 T/A) genotypes was significantly different in controls and cases ($P=.001$, OR 1.603, 95\% CI 1.032-2.489). The frequencies of \textit{DRD2} (Ser311Cys) SS, SC, and CC genotypes were 46.2\%, 43.2\%, and 10.6\% in cases and 60.7\%, 32.1\%, and 7.2\% in controls, respectively. The distribution of \textit{DRD2} (Ser311Cys) genotypes was significantly different in controls and cases ($P=.001$, OR 1.83, 95\% CI 1.258-2.6). No association was observed in the polymorphism of the +61450 C/A of \textit{GAD2} gene. The allelic distribution of \textit{GAD2} (+83897 T/A) and \textit{DRD2} (Ser311Cys) polymorphisms between the two groups were statistically significant ($P=.034$ and $P=.036$), respectively.

\textbf{DISCUSSION}

Obesity is a chronic disease that contributes to metabolic complications, including hypertension, cardiovascular disease, type 2 diabetes, and some cancers in both men and women. These complications increase the risk of mortality and morbidity worldwide. Genetic risk factors play an important role in the development of obesity in humans. Rankinen et al\textsuperscript{23} described an obesity-related genes map to show putative loci on all chromosomes except Y. A total of 127 candidate genes have been reported, and these gene variations were associated with obesity phenotypes. Among these genes, Blum et al\textsuperscript{24} found that low D2 receptor density and \textit{DRD2} gene polymorphisms were associated with risk for relapse of substance abuse, including alcohol dependence, heroin craving, cocaine dependence, methamphetamine abuse, nicotine sensitization, and glucose craving. Moreover, the defect of the \textit{DRD2} gene is associated with RDS, which is a dysfunction in the brain reward cascade involved in abnormal craving behavior. In spite of all the supportive data, few genetic treatments have not been developed to attenuate the abnormal craving behaviors. Blum et al\textsuperscript{25} have succeeded in the development of the DNA-customized nutraceutical product, LG839, which is an antiobesity agent, that could increase weight loss, decrease food cravings, prevent weight regain, and also reduce stress. Among the obesity-related genes (LEP, \textit{PPAR-$\gamma$2}, MTHFR, \textit{5-HT2A}, and \textit{DRD2} genes), only the \textit{DRD2} genes polymorphism had a significant association with days on treatment.

\textbf{Table 1. Demographic data of cases and controls.}

| Group   | N  | Body mass index (kg/m$^2$) | Age (years) | Women (%) |
|---------|----|---------------------------|-------------|-----------|
| Cases   | 132| 38.2 (7.4)                | 48.9 (10.9) | 75        |
| Controls| 168| 21.5 (2.3)                | 56.8 (13.4) | 70        |

Values are mean and standard deviation unless otherwise indicated.
DA is a neurotransmitter in the brain reward pathway that controls feelings of motivation, reward, and behaviors through the interaction with D2 receptor. The central job of the reward pathway is to make us feel better while we are involved in behaviors that are necessary for our survival. These beneficial behaviors include eating, drinking, and sex. Tatarrani et al described that the absence of the murine dopamine D2 receptor gene led to the bradykinesia and hypothermia. These findings were the first evidence indicating that a genetic mutation was associated with reduced energy expenditure in humans. The authors suggested that the impact of this mutation on human obesity was small, and that the energy deficit induced was not large enough to significantly influence body weight in this population. Southon et al described that Ser311Cys polymorphisms in the DRD2 gene are unlikely to be common causes of obesity in the Nauruan and Australian population.

GABA is also a neurotransmitter in the mammalian central nervous system. It plays a major role in enhancing food intake by interacting with neuropeptide Y (NPY). Many other physiologic processes in the brain are also associated with NPY, including the regulation of energy balance, memory, and learning; epilepsy is also associated with NPY. Allen et al discovered the highest levels of NPY immunoreactivity within the paraventricular nucleus of the hypothalamus in the rat brain. With in situ hybridization and immunoassay studies, Hanson et al observed that the NPYergic activity increases the food intake of rats. This was further confirmed by behavioral assays. Additionally, orexigenic studies have proved that exogenous NPY could also enhance feeding behavior, such as a NPY agonist: dexamethasone or N-acetyl (Leu28, Leu31) NPY (24-36). Moreover, Dryden et al reported that weight gain was increased by the hypothalamic arcuate nucleus.

Table 2. Genotype and allele distributions among the cases and controls.

| Genotype | Cases N, (%) | Controls N, (%) | OR (95% CI) | P |
|----------|--------------|-----------------|-------------|---|
| GAD2 +61450 C/A | Genotype | | | |
| CC       | 60 (45.5%)   | 72 (42.9%)      | 0.986 (0.707-1.376) | NS |
| CA       | 42 (31.8%)   | 64 (38.1%)      |             |   |
| AA       | 30 (22.7%)   | 32 (19.0%)      |             |   |
| Allele   |             |                 | 0.898 (0.616-1.303) | NS |
| C        | 162 (61.4%)  | 208 (61.9%)     |             |   |
| A        | 102 (38.6%)  | 128 (38.1%)     |             |   |
| GAD2 +83987 T/A | Genotype | | | |
| TT       | 42 (31.8%)   | 25 (14.9%)      |             |   |
| TA       | 61 (46.3%)   | 89 (52.9%)      |             |   |
| AA       | 29 (21.9%)   | 44 (26.2%)      |             |   |
| Allele   |             |                 | 1.603 (1.032-2.469) | .001 |
| T        | 145 (54.9%)  | 139 (41.3%)     |             |   |
| A        | 119 (45.1%)  | 177 (52.7%)     |             |   |
| DRD2 Ser311Cys | Genotype | | | |
| Ser/Ser  | 61 (46.2%)   | 102 (60.7%)     |             |   |
| Ser/Cys  | 57 (43.2%)   | 54 (32.1%)      |             |   |
| Cys/Cys  | 14 (10.6%)   | 12 (7.2%)       |             |   |
| Allele   |             |                 | 1.913 (1.621-2.375) | .034 |
| Ser      | 179 (67.8%)  | 258 (76.8%)     |             |   |
| Cys      | 85 (32.2%)   | 78 (23.2%)      |             |   |

OR: Odds ratio, NS: not significant, Ser: serine, Cys: cysteine.
and the paraventricular nucleus pathway in the fatty Zucker rat, a well-known animal model of obesity and insulin resistance. The results support the association between NPY and obesity.

A recent study investigated a role of positional candidate gene GAD2, which encodes for glutamate decarboxylase, in the development of morbid obesity. Previous reports have found 15 variants in a coding and regulating region in patients with type 1 diabetes. In a study reported by Boutin et al., 3 variants -243 A/G, +61450 C/A, and +83897 T/A in GAD2 were found, and these variants were associated with obesity in the French population. The activity of the promoter variant -243 A/G in GAD2 was about 6 times higher than that of the wild-type promoter, and it could increase the concentration of GABA and increase dietary intake in the hypothalamus. Additionally, the +61450 C/A and +83897 T/A variants in coding region of GAD2 have proved the implications of family history of obesity in obese patients. Likewise, 6 GAD2 sequence variants were genotyped by Choquette et al. The results proved the association between +61450 C/A and +8473 A/C polymorphisms and eating behavior and dietary intake in women. The two variants could significantly increase BMI and body weight. The authors suggested these GAD2 polymorphisms influence eating behavior and dietary intake, resulting in increased weight gain. In the current study, a significant association was found between +83897 T/A polymorphism and dietary intake. We did not observe any association between the GAD2 -243 A/G variant and obesity in this study. In the future, we will conduct an adequately powered case-control study to test the association between obesity and the GAD2 -243 A/G variant in Taiwanese. This case-control study will help in a meta-analysis of the findings of other research performed to study the association between the -243 A/G and obesity.

In conclusion, these experiments suggest that the polymorphisms of GAD2 (+83897 T/A) and DRD2 (-243 A/G) are significantly associated with an increased risk of developing obesity. We recognized that the value of this study was limited by a relative small sample and also that only a few variants in each gene were studied. Eventually, if confirmatory studies are done on large populations to assess the significance, these variants may be concluded as factors predisposing an individual to obesity.

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