INTRODUCTION

Obesity is one of the most common non-communicable diseases and public health issues in the world at every stage of development. The World Health Organization (WHO) estimates that 13% of adults worldwide are obese ***(World Health Organization, 2016).*** Obesity is associated with increased risk of comorbid diseases such as cardiovascular diseases, diabetes mellitus, hypertension, musculoskeletal disorders especially osteoarthritis and some cancers as breast, endometrial, ovarian, prostate, liver and colon. Moreover; a higher mortality rate was recorded among obese adults as a consequence of the associated comorbidities ***(Flegal et al., 2013).***

Genome-wide scans for obesity-associated genes have repetitively detected a linkage with a locus on chromosome 10p12.1. The previous studies showed that glutamic acid decarboxylase 2 *(GAD2)* gene is one of the genes located in this locus, where it encodes for the GAD2 enzyme (GAD65) ***(Prakash et al., 2015).***

The enzyme GAD is involved in the hypothalamic regulation of food intake by controlling the formation of the γ-aminobutyric acid (GABA) from the glutamic acid, where the former functions together with neuropeptide Y in the paraventricular nucleus to stimulate food intake ***(Matthew et al., 2015).***

Some researchers hypothesize that single nucleotide polymorphisms (SNPs) in *GAD2* gene may enhance the over expression of the GAD2 resulting in overeating and obesity. One of the suggested SNPs in *GAD2* gene is the -243A>G polymorphism (rs2236418) in which the adenine (A) is substituted by guanine (G) at the 243 position in the 5-prime promoter region of the *GAD2* gene. Some recent studies started to explore the association between this polymorphism and the enhanced transcriptional activity of *GAD2* gene ***(Prakash et al., 2015).***

AIM OF THE WORK

The aim of the present study is to investigate the relationship between -243A>G polymorphism of *GAD2* gene and the presence of obesity.

Chapter I

OBESITY

A) Epidemiology

World Health Organization (WHO) studies (2016) revealed that the worldwide prevalence of obesity nearly tripled between 1975 and 2016. The studies demonstrated that about 13% of the adult population worldwide were obese and 39% were overweight ***(World Health Organization, 2016).***

In developing countries, the average weight gain is greater than that observed in developed countries among the adults. Prevalence of obesity in developing countries ranges from 2.3 to 12%, with overweight as high as 28.8% ***(Poobalan and Aucott, 2016).***

Obesity is highly prevalent in Egypt. The estimated prevalence of overweight and obesity is 61–70% of the adult population aged 20 and above, with a percentage of 65% of males and 76% of females. Obesity is also considered as one of the top contributors to the mortality of Egyptians along with other non-communicable diseases such as hypertension and diabetes that are responsible for 41% of all mortality in Egypt ***(Abdelmajed et al., 2017).***

B) Definition and Classifications of Obesity

Overweight and obesity are defined as an abnormal excessive fat accumulation that may impair health. Based on body mass index (BMI), the adult body weight can be classified into normal weight, with BMI between 18.5 and 24.9 kg/m², class I obesity or overweight with BMI between 25 and 29.9 kg/m², class II obesity with BMI between 30 and 39.9 kg/m² and class III or morbid obesity with BMI between 40 and 59 kg/m² ***(Kitahara et al., 2014).*** However, BMI does not discriminate directly between muscle and adipose tissue and does not assess regional obesity ***(Panigrahi et al., 2009).***

Another classification based on fat distribution measurement using waist circumference and waist-to-hip ratio is more reliable in determining the risk for cardiovascular diseases and diabetes mellitus type 2. A waist circumference >101.6 cm in men and >88.9 cm in women or a waist-to-hip ratio >1 in men and >0.85 in women specifies android or visceral obesity and is closely associated with comorbidities ***(Zhang et al., 2016).*** Meanwhile, gynoid obesity which is associated with lower fat distribution in hips, femoral and gluteal region has the properties of "metabolically healthy obesity" ***(Pinkhasov et al., 2016).***

The concept of "Normal Weight Obesity" has also emerged in the past few years. It is defined as normal BMI between 18.5 and 24.9kg/m² (normal weight), but the sum of

triceps and subscapular skin folds is >90th percentile, which corresponds to >23.1% body fat in men and >33.3% body fat in women ***(Poobalan and Aucott, 2016).***

C) Homeostatic Regulation of Body Weight

The homoeostatic regulation of body weight is responsible for energy balance through the regulation of energy intake as well as the energy expenditure ***(Moehlecke et al., 2016).***

1- Regulation of energy intake

The regulation of energy intake is based on the control of appetite and food intake. The process is derived from a variety of afferent signals received from peripheral locations and from neighboring regions in the brain. These afferent signals are processed in the central nervous system (hypothalamus and brain stem). The subsequent release of efferent signals from the limbic cortex, thalamus, brain stem, insula, hippocampus and base nuclei regulates the appetite and satiety ***(Moehlecke et al., 2016).***

Three types of afferent signals are recognized based upon their location:

a) Central signals:

The arcuate nucleus (ARC) of the hypothalamus is the primary brain region involved in the homoeostatic control of food intake. The ARC contains two distinct interconnected

groups of neurons with opposing effects on energy balance. Neurons which co-express orexigenic neuropeptides as neuropeptide Y (NPY), agouti-related peptide (AgRP) and γ-aminobutyric acid (GABA) function together to stimulate the appetite ***(Atasoy et al., 2012 and Matthew et al., 2015).***

Meanwhile, neurons expressing anorexigenic peptides as pro-opiomelanocortin (POMC), cocaine and amphetamine-related transcript (CART) decrease food intake and increase energy expenditure and weight loss via the release of alpha-melanocyte- stimulating hormone (MSH) with subsequent activation of melanocortin receptors ***(Velloso et al., 2009).*** The melanocortin-4 receptor (MC4R) is the primary receptor expressed in the hypothalamus and is a part of the melanocortinergic pathway ***(Tao, 2010).***

In addition, two neuropeptides are expressed by neurons in the lateral hypothalamus; the melanin-concentrating hormone (MCH) and orexins where both were found to stimulate the appetite ***(Sumithran and Proietto, 2013).***

b) Post-prandial signals:

These signals are released during and after meal intake. They include peptides from gastrointestinal tract and pancreas such as:

- Gherlin which stimulates the release of pancreatic digestive enzymes

- Insulin which suppresses the expression of the orexigenic AgRP and the NPY and promotes the POMC –derived peptide production ***(Cowely et al., 2003).***
- Glucagon –like peptide-1 (GLP-1) which stimulates insulin release
- Peptide YY (PYY) released from the neuroendocrine cells in the ileum and colon to inhibit gastric motility and pancreatic secretions.

All these hormones decrease appetite except for gherlin which increases the appetite ***(Sumithran and Proietto, 2013).***

c) Adipose tissue signals:

These signals depend on the production of bioactive proteins from the adipose tissue referred to as "adipokines" which include leptin, adiponectin and resistin ***(Moehlecke et al., 2016).***

- Leptin secretion is stimulated by insulin and is involved in the long-term regulation of energy balance by acting in the hypothalamus to reduce food intake and increase energy expenditure by reducing the expression of AgRP, NPY, and stimulating that of POMC, orticotrophin releasing hormone (CRH) and CART ***(Sumithran and Proietto, 2013).***

- Adiponectin increases the sensitivity to insulin in muscles and hepatocytes, reduces the hepatic glucose production and stimulates fatty acid oxidation ***(Moehlecke et al., 2016).***

- Resistin reduces insulin- stimulated glucose uptake in isolated adipocytes. Elevated resistin levels are associated with greater risk for type 2 diabetes mellitus, increased inflammatory markers and atherosclerosis ***(Moehlecke et al., 2016).***

2- Regulation of energy expenditure

Energy expenditure is performed by three different mechanisms; obligatory thermogenesis (resting metabolic rate of energy expenditure), post-prandial thermogenesis and thermogenesis resulting from physical activity either exercise or non-exercise activity (activities as muscle contractions, maintaining posture and involuntary movements) ***(Ricquier, 2006).***

Several mediators are involved in the modulation of energy expenditure:

a) Autonomic neurotransmitters:

Serotonin and norepinephrine are autonomic neurotransmitters which reduce food intake and increase sympathetic activity ***(Moehlecke et al., 2016).***

b) Thyroid hormones:

Thyroid hormones have been demonstrated to modulate many metabolic pathways potentially relevant for the basal metabolic rate like glycolysis, gluconeogenesis and lipolysis and could also increase the protein turnover ***(Bianco et al., 2005).***

c) Irisin:

Irisin is secreted by monocytes in response to exercise and appears to mediate the beneficial effects of exercise on metabolism by stimulating the transformation of white adipose tissue into brown adipose tissue, an effect known as "browning" of subcutaneous adipose tissue, by increasing the expression of uncoupling protein 1 (UCP1, thermogenin). This effect leads to the increase of energy expenditure with reduction of body weight and the incidence of type 2 diabetes mellitus ***(Bostrom et al., 2012).***

d) Energy balance- related peptides:

They include gherlin and MCH which were found to decrease energy expenditure, leptin and CART which increase energy expenditure ***(Moehlecke et al., 2016).***

D) Etiology and Mechanisms of Obesity

Obesity is a chronic multifunctional disorder resulting from disruption of the balance between energy intake and

energy expenditure. The disease has a complex etiology involving behavioral, hormonal and genetic factors ***(Moehlecke et al., 2016).***

1- Behavioral factors:

Behavioral factors favoring a positive energy balance and weight gain include increase food intake, consumption of sugar sweetened beverages, sweet snacks and fast foods, decrease time of physical activities and displacement of leisure-time physical activities with sedentary activities such as watching television and the use of electronic devices ***(Apovian et al., 2015 and Popkin and Hawkes, 2016).***

2- Neuroendocrinal factors:

Neuroendocrinal factors include endocrinal and acquired hypothalamic lesions ***(Kumar and Kelly, 2017).***

a) Endocrinal dysregulation:

Several endocrinal dysregulations can lead to an imbalance in metabolism and interference in hormonal control of adipose tissue functions with subsequent inappropriate deposits of fat and hence, obesity **(Figure 1)** ***(Karam and McFarlane, 2007).***

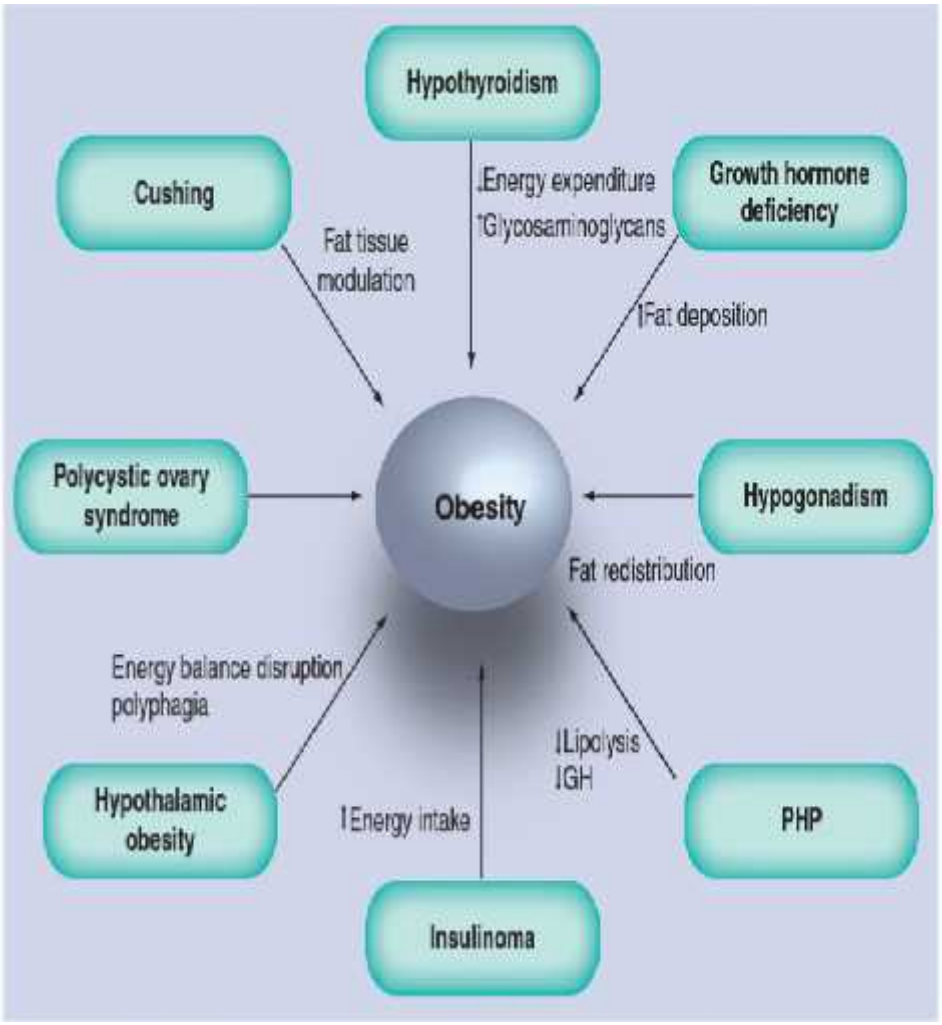

Figure (1): Endocrinologic etiologies of obesity and related pathophysiology. PHP: Pseudohypoparathyroidism; GH: Growth hormone ***(Karam and McFarlane, 2007).***

Cushing syndrome results from prolonged exposure to glucocorticoid excess derived from four potential sources; pituitary tumors (Cushing disease), adrenal tumors, ectopic adreno-corticotropic hormone (ACTH) secretion and exogenous glucocorticoids ***(Karam and McFarlane, 2007).*** The disease leads to weight gain by enhancing fat tissue

modulation, particularly central adiposity with fat accumulation in the face (moon face), neck, dorsocervical (buffalo hump), supraclavicular area (fat pads), trunk and abdomen with sparing or wasting of extremities by decreasing the peripheral adipose tissue mass. It also inhibits the suppressive action of leptin on food intake and body weight and antagonizes the secretion and action of insulin which leads to secondary diabetes ***(Karam and McFarlane, 2007 and Sumithran and Proietto, 2013).***

Hypothyroidism, hypogonadism, polycystic ovaries, insulinoma, pseudo-hypoparathyroidism and growth hormone deficiency are other endocrinal disorders causing weight gain and obesity ***(Kumar and Kelly, 2017).***

b) Acquired hypothalamic lesions:

Acquired hypothalamic lesions such as craniopharyngioma, particularly after surgery and/or cranial radiation can present with weight gain. Patients may have symptoms of increased intracranial pressure such as headache and vomiting and may also have symptoms of panhypopituitarism. Weight gain may also be seen in patients after cranial trauma or inflammatory disease affecting the hypothalamus ***(Kumar and Kelly, 2017).***

3- Genetic factors:

Large-scale genetic studies confirm the involvement of genetic variants involved in obesity and also highlight the role of genes expressed in the brain in the pathogenesis of obesity,

suggesting its strong link to eating behavior. Genetic factors could be heritable or acquired where the heritable factors appear to be responsible for 30% to 50% of obesity while the acquired genetic factors can be due to changes in gene transcription and translation through environmental influences. Epigenetic effects, which are external modifications in the DNA that turn genes “on” or “off” without changing the DNA sequence can also occur, in the form of DNA methylation and histone modification ***(Bray et al., 2016).***

A distinction is often made between three types of obesity based on genetic basis: monogenic, syndromic, and polygenic forms of obesity, where the latter accounts for the most commonly observed cases ***(Wood and Wang, 2016).***

a) Monogenic forms of obesity

Monogenic obesity is due to mutations within a single gene which is transferred from parent to offspring. It has discrete causes and discrete consequences which are not present in the non- obese and so it is referred to as “qualitative inheritance”. The two alleles of this gene are located in the same locus. It is associated with severe and pervasive obesity in the absence of environmental factors ***(Wood and Wang, 2016).***

Melanocortin 4 receptor gene *(MC4R)* mutations are the most common cause of monogenic obesity, being the primary cause seen in over 6 % of the obese individuals. Prepubertal,

the extent of functional impairment in MC4R signaling, correlates positively with obesity through a hyperphagic behavior and a reduced metabolic rate ***(Wood and Wang, 2016).***

Leptin gene mutation is a rare form of monogenic obesity that may lead to a complete lack of circulating leptin. It leads to a severe early-onset obesity with hyperphagia alongside additional endocrine abnormalities such as insulin resistance and type 2 diabetes mellitus maybe present ***(Dubern and Clement, 2012 and Tong et al., 2017).*** Obesity arising from leptin deficiency is one of the few forms of obesity with effective therapeutic options. Daily lifelong injections of leptin result in a reduction in fat mass attributed mostly to treating the hyperphagic aspect of leptin deficiency ***(Dubern and Clement, 2012).***

Mutation in the leptin receptor gene is another rare cause of obesity present only in 3% of obese patients. Affected individuals produce leptin, but the receptor binds to leptin, preventing its uptake, leading to extremely high serum leptin levels. Similar to patients with leptin gene mutation, those with leptin receptor mutation-dependent obesity exhibit severe hyperphagia and endocrine abnormalities as type 2 diabetes mellitus. This form of monogenic obesity has no treatment ***(Dubern and Clement, 2012).***