Abstract

STUDY OF THE COMMON VARIANT RS9939609 OF FTO GENE POLYMORPHISM IN POLYCYSTIC OVARY SYNDROME

Polycystic Ovarian Syndrome (PCOS) is the most common endocrine abnormality in reproductive-age women. Characterized by menstrual disturbances, clinical and biochemical manifestations of hyperandrogenism and polycystic ovaries

PCOS frequently coexists with obesity and type 2 diabetes mellitus. The evidence from family-based and unrelated association studies suggests that obesity and PCOS have a significant inherited basis, pointing to a shared genetic predisposition in contributing to their co-occurrence.

Fat mass and obesity-associated (FTO) gene is located on chromosome 16 and expressed in a wide range of tissues, including the adipose tissue and specific areas of the brain and muscles, suggesting its potential role in body weight regulation

The variant FTO rs9939609, located within the first FTO intron has two alleles, A and T, the former has been linked to an increased risk for both obesity and type 2 diabetes mellitus

In view of the previous facts, the present study investigated the association between the common variant rs9939609 of FTO gene with polycystic ovary syndrome in Egyptian women.

For this purpose samples were collected from twenty five patients diagnosed as PCOS according to the criteria of Rotterdam Revised (2003) (Group I) and twenty five (25) age-matched healthy females (Group II)

All individuals in this study will be subjected to the following Full history taking, physical examination including height and weight to calculate Body Mass Index (BMI), Pelvic Ultrasound and Lab investigations including Serum total testosterone, Follicle stimulating Hormone (FSH), Luteinizing Hormone (LH) and prolactin, Fasting plasma glucose, fasting Insulin, Homeostasis Model Assessment of Insulin Resistance (HOMA IR) was calculated as well as Detection FTO rs9939609 Polymorphism by real time Polymerase Chain Reaction (PCR).

This study revealed a significant difference between PCO patient group and control group as regards the of FTO genotypes A/A, A/T and T/T. An increase in frequency of A/T and T/T genotypes in PCO patient group (56% A/T and 8% T/T) compared with control group (0%) on the other hand control group individuals showed A/A genotype only.

In conclusion, the common rs9939609 variant in FTO gene is associated with PCO in Egyptian women.

Keywords: *FTO, Single nucleotide polymorphism, rs9939609, PCO*

Introduction

Polycystic Ovarian Syndrome (PCOS) is the most common endocrine abnormality in reproductive-age women. PCOS prevalence is estimated to be 5-10% in different ethnic populations and 22% of women in general population have polycystic ovaries on ultrasound. It is a heterogeneous disorder, characterized by menstrual disturbances, clinical and biochemical manifestations of hyperandrogenism and polycystic ovaries ***(Gersak and Ferk, 2007 & Allahbadia and Merchant, 2011).***

The etiologies of PCOS have not been fully elucidated. It frequently coexists with obesity and insulin resistance. It is reported that approximately 50% of PCOS women are overweight or obese. This implies that obesity is an important factor in the etiology of PCOS. The evidence from family-based and unrelated association studies suggests that obesity and PCOS have a significant inherited basis, pointing to a shared genetic predisposition in contributing to their co-occurrence. Both PCOS and obesity are considered highly heritable complex diseases ***(Wehr et al., 2010).***

Discovery of fat mass and obesity-associated (FTO) gene was the first major success in the field of obesity genetics ***(Frayling et al., 2007 & Scuteri et al., 2007).*** The human FTO gene is located on chromosome 16 and expressed in a wide range of tissues, including the adipose tissue and specific areas

of the brain and muscles, suggesting its potential role in body weight regulation ***(Wehr et al., 2010).***

The FTO gene is highly polymorphic, and several polymorphisms of the gene have been described. The variant FTO rs9939609, located within the first FTO intron has two alleles, A and T alleles ***(De Luis et al., 2013).***

Several studies were set out to establish whether FTO variants impact on the individual risk of PCOS, however controversial results were obtained among different ethnic population.

Aim of the Work

The aim of the present study is to investigate the association between the common variant rs9939609 of FTO gene with polycystic ovary syndrome in Egyptian women.

I - Polycystic Ovarian Syndrome

Polycystic ovary syndrome (PCOS) is a complex endocrine metabolic disease affecting 6–8% women of reproductive age. It is characterized by clinical and/or biochemical androgen excess, ovulatory dysfunction and polycystic ovaries ***(Li et al., 2013).***

Family-based and case-control association studies suggest genetic factors contribute to both obesity and PCOS, which implicates a shared genetic predisposition in their concurrence ***(Walley et al., 2006 and Li et al., 2013).***

A. Definition:

As defined by the Rotterdam criteria, two of the following three criteria are associated with polycystic ovary syndrome (PCOS): oligo-anovulation, ultrasonographically defined polycystic ovaries and clinical or biochemical signs of hyperandrogenism without any other disorders that cause androgen excess ***(Rotterdam 2003 and Deveci et al., 2014).***

B. Prevalence in Egyptian Women:

In most studies, the prevalence of PCOS in reproductive age women is estimated to be between 5-10%, but the prevalence rates reported are naturally dependent on the ethnicity of the studied population ***(March et al., 2010).***

A prospective analysis was conducted on a cohort of 3,900 Egyptian patients seeking fertility advice at a specialized fertility clinic. The prevalence of confirmed PCOS cases among this cohort had been estimated to be 10.48% ***(Saleh and Shawky, 2014).***

C. Etiology:

The etiology of PCOS is controversial and more than a factor is involved in development and disease progression**.** Data are accumulating that PCOS is a complex trait with contributions from both heritable and environmental factors. (Figure 1) ***(McCarthy et al., 2012)***

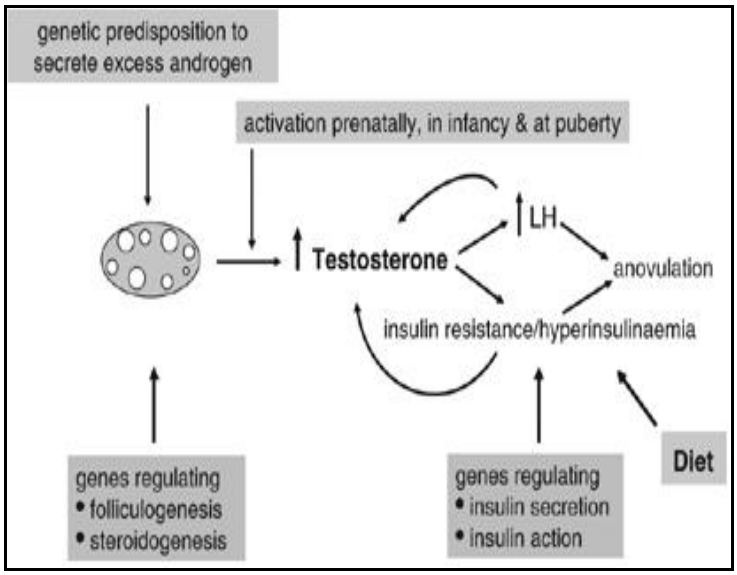

Figure (1): Proposed developmental etiology of polycystic ovary syndrome (PCOS) ***(McCarthy et al., 2012)***

Three popular theories was proposed in different studies considering etiology of PCOS; The first theory suggests a direct

correlation between high levels of circulating luteinizing hormone (LH) cause and increase in the growth of theca interstitial cell (TIC) in developing follicles, and this might cause androgen increase and follicular atresia ***(Dasgupta and Reddy, 2013).***

The follicle stimulating hormone-granulosa cell (FSH-GC) theory, the second theory, suggests that the low levels of FSH causes subnormal induction of cytochrome P450 aromatase in the granulosa cells, leading to an increase of androgen levels. This may be due to insufficient bioactive FSH in the follicular microenvironment to induce P450 aromatase gene expression, dysfunctional FSH receptor signal transduction mechanism, or the presence of inhibitors (such as epidermal growth factor and insulin-like growth factor (IGF)-binding protein 3, that prevent the normal expression of P450 aromatase activity ***(Dasgupta and Reddy, 2013).***

The third theory is related to the growth factor-autocrine-paracrine system. In PCOS, there is evidence of an altered IGF/insulin system, and these act as mediators of biologic responses of follicular hormones ***(Dasgupta et al., 2013).***

D. Pathogenesis:

The most common biochemical abnormality in women with PCOS is hyper secretion of androgen. The ovary appear to be the predominant source of excess androgen production ***(Dasgupta and Reddy, 2013).***

Overproduction of ovarian androgen in PCOS syndrome results from abnormalities at all levels of the hypothalamic pituitary ovarian axis. The increased frequency of LH pulses in the PCOS appears to result from an increased frequency of hypothalamic gonadotropin-releasing hormone (GnRH) pulses. The latter can result from an intrinsic abnormality in the hypothalamic GnRH pulse generator, favoring the production of luteinizing hormone over follicle-stimulating hormone (FSH) in patients with PCOS, in whom the administration of progesterone can control the rapid pulse frequency ***(Ehrmann, 2012).***

The relative increase in pituitary secretion of LH leads to an increase in androgen production by ovarian theca cells. Increased efficiency in the conversion of androgenic precursors in theca cells leads to enhanced production of androstenedione which is then converted by 17p-hydroxysteroid dehydrogenase (17P-HSD) to form testosterone or aromatized by the aromatase enzyme to form estrone. Within the granulosa cell, estrone is the converted into estradiol by17p-HSD. (Figure 2) ***(Dosgupta and Reddy, 2013)***

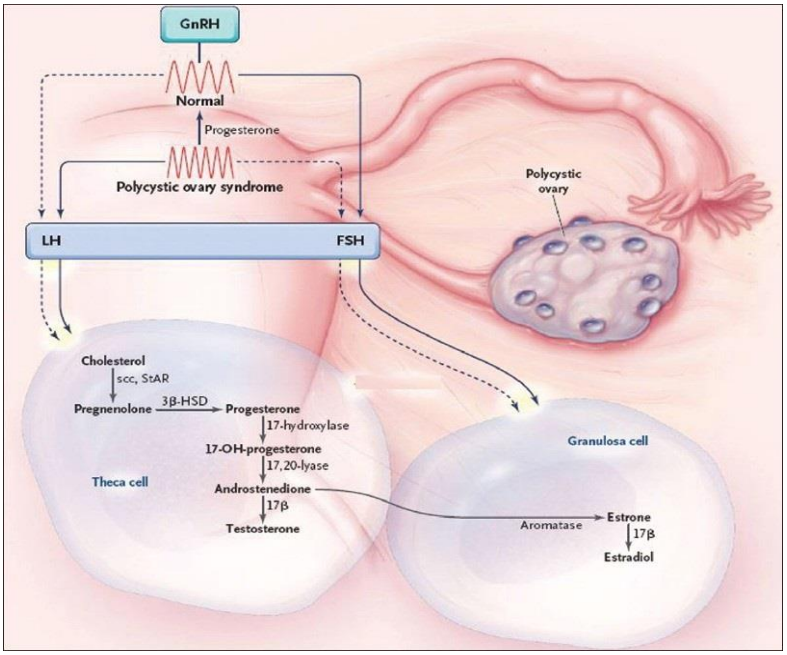

Figure (2): Hypothalamic-pituitary ovarian axis in PCOS ***(Dosgupta and Reddy, 2013)***

SCC*: Side chain cleavage enzyme,* ***STAR****: steroidogenic acute regulatory protein and 3β-HSD: 3β hydroxy steroid dehydrogenase.* ***Solid arrows****: higher degree of stimulation than dashed arrows*

Outward signs of the disease result from hyperandrogenism secondary to overproduction of testosterone from ovarian theca cells and/or adrenals. Increased androgens are responsible not only for hirshutism and acne, but also play a role in promoting abnormal follicular development leading to menstrual disturbances. Even a mild, chronic elevation in LH can blunt the effect of the hormone surge, leading to anovulation ***(Goodman et al., 2015).***

Women with PCOS, both lean and obese, may be insulin resistant. This is thought to be due to a post-receptor defect affecting glucose transport ***(Homburg, 2008***). The presence of central obesity may aggravate insulin resistance, further worsening the hormonal imbalance. Generally, the degree of insulin resistance is mild, although the prevalence of glucose intolerance and subsequent diabetes has been reported to be as high as 31% and 7.5%, respectively. In addition, there is indirect evidence to indicate that insulin resistance may worsen the clinical manifestations of PCOS. Administration of insulin-lowering drugs has been shown to improve insulin sensitivity, reduce androgen levels, and restore ovulation in some, but not all patients with this disorder. Insulin resistance may also contribute to metabolic dysfunction in PCOS, including an increased likelihood of lipid abnormalities. Moreover, insulin acts on the liver, adrenal, ovary, and pituitary to increase circulating free androgen. Increased free androgen, in turn, may increase visceral adiposity (Figure 3) ***(Chang, 2004; Takara and Madhusmita, 2011).***

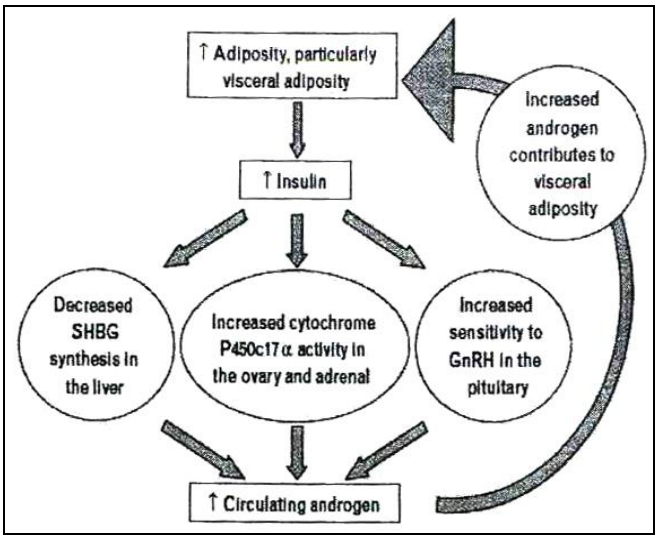

Figure (3): Insulin effect in PCOS ***(Takara and Madhusmita, 2011)***

In patients suffering from PCOS, the incidence of obesity is somewhere between 50%: 74% which is higher than the general population ***(Helmrath et al., 2006)***. Obesity is more common among women with PCOS, obesity may exacerbate many of the manifestations of PCOS including androgen levels and insulin resistance. With excess weight gain, women who were previously asymptomatic may begin to show symptoms of PCOS ***(Hoeger and Operfiald, 2012).***

As compared to non-obese PCOS women, obese women with PCOS have more menstrual irregularities and uterine dysfunctional bleeding, as well as an increased prevalence of infertility, which were also associated with an abdominal distribution of fat. Obese PCOS women also have a higher risk of developing glucose intolerance or diabetes than lean PCOS women ***(Baptiste et al., 2010).***

E. Diagnosis of PCOS:

1. Diagnostic criteria

Three guidelines can be followed for PCOS diagnosis; the first is the National Institute of Child Health and Human Development (NICHD), and the second is conference Human Reproduction and Embryology (ESHRE)/American Society for Reproductive Medicine (ASRM). The third one was introduced by the Androgen Excess Society, which consider both the criteria existent till date as shown in (Table 1) ***(Karger et al., 2013).***

Table (1): A comparison of diagnostic criteria for PCOS

1990 National Institute of Child Health and Human Development (NICHD) Guidelines	Patient demonstrates both: 1. Clinical and/or biochemical signs of hyperandrogenism. 2. Oligo- or chronic anovulation. • Exclusion of other etiologies of androgen excess and anovulatory infertility is necessary.
2003 European Society for Human Reproduction and Embryology and American Society for Reproductive Medicine (ESHRE/ASRM or Rotterdam) Guidelines	Patient demonstrates two of three criteria: 1. Oligo- or chronic anovulation 2. Clinical and/or biochemical signs of hyperandrogenism 3. Polycystic ovaries • Exclusion of other etiologies of androgen excess and anovulatory infertility is necessary.
2006 Androgen Excess Society (AES) Guidelines	Patient demonstrates both: 1. Hirsutism and/or hyperandrogenemia 2. Oligo-anovulation and/or polycystic ovaries • Exclusion of other etiologies of androgen excess and anovulatory infertility is necessary.

(Karger et al., 2013)

2. Diagnostic approach

When the combination of chronic anovulation and androgen excess is used as a working definition of PCOS, the clinician will avoid making the wrong diagnosis. First, with respect to ovulatory history, women with PCOS classically give a history of irregular menstrual cycles dating to menarche. As a role of thumb, they generally report 6 or fewer episodes of spontaneous vaginal bleeding per year. A women with history of cyclic, predictable menstrual cycles followed by secondary amenorrhea, however, specially is absence of androgen excess, probably does not have PCOS. These individuals should be evaluated for other causes of amenorrhea, beginning with thyroid stimulating hormone, prolactin and FSH ***(Glaeuck et al., 2012).*** The clinical features of PCOS is summarized in (Table 2) ***(Rosenfield et al., 2011).***

Table (2): Clinical features of PCOS

Cutaneous manifestations of hyperandrogenism	- Hirsutism - Acne
Ovarian Findings	- Anovulation - Polycystic ovaries
Associated metabolic features	- Obesity - Manifestations of insulin resistance
Menstrual disorders.	- Amenorrhea - Oligomenorrhea

(Rosenfield et al., 2011)

3. Laboratory Diagnosis

a. ***Serum LH and FSH:***

Increased serum LH typically > 2.5 times the FSH. Although it is a useful marker of the syndrome, it is less favored as a diagnostic tool ***(Dunaif, 2012).*** This is because LH levels are increased in approximately 40- 70% of women who fit clinical criteria for PCOS ***(Fauser et al., 2012).***

The secretion of LH occurs in pulsatile fashion as a result of pulsatile nature of gonadotropins, limiting the reliability of a single measurement. Furthermore, recent ovulation, which occurs sporadically in PCOS women, can normalize observed LH levels for 2-3 weeks after ovulation ***(Van Hoffet al., 2010).*** Also, BMI (Body Mass Index) correlates negatively with LH pulse amplitude and basal LH levels in women with PCOS, limiting its use in overweight women ***(Taylor et al., 2012).***

b. ***Serum total testosterone:***

Elevated serum total testosterone level due to increased androgen production in theca cells ***(Dunaif, 2007).***

c. ***Fasting insulin measurement***:

Testing for insulin resistance has become an important part of the evaluation, because 35% to 45% of PCOS patients have impaired glucose tolerance, including 7% to 10% with type-2 diabetes ***(Ehrmann et al., 2012).*** Insulin resistance is assessed by Homeostasis Model Assessment of Insulin Resistance (HOMA IR)

HOMA-IR= [Glucose in mg/dl,] X [Insulin in μU/mL] / 405.

II - Fat Mass and Obesity Associated Gene

A. Discovery of *FTO* Gene

FTO gene was originally identified in 1999 as one of six contiguous genes on chromosome 8 in a mouse model known as fused toes (Ft). In addition to *FTO*, the other five genes include Irx3, Irx5, Irx6, Fts, and RPGRIP1L ***(Peters et al., 2010).*** The genomic region adjacent to *FTO* together with the relative location of these other genes are shown in (Figure 4) ***(Cheunug and Yeo, 2011)***

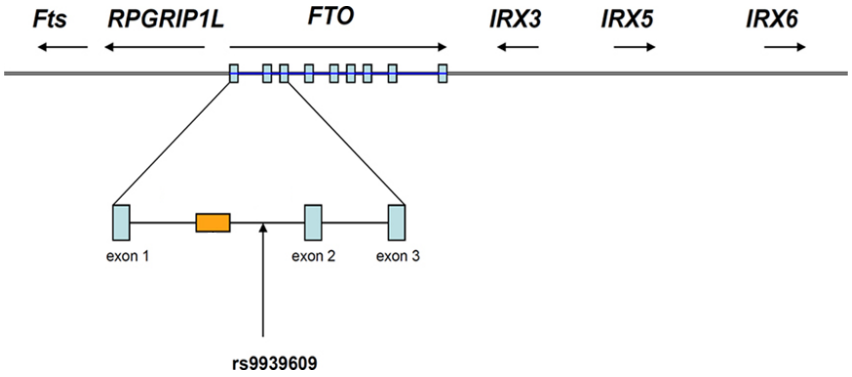

Figure (4): Genomic organization of *FTO* and its neighboring genes ***(Cheunug and Yeo, 2011)***

The *FTO* gene contains nine exons spanning more than 400 Kb on chromosome no. 16 in human which are depicted in the blue rectangles. The most replicated *FTO* single nucleotide polymorphism (SNP) rs9939609 is found in intron 1, the first and the largest of the gene ***(Loos et al., 2008) & (Stratigopoulos et al., 2011).***