ASSOCIATION BETWEEN POLYMORPHISMS OF THE CYP11A1 GENE AND POLYCYSTIC OVARY SYNDROME IN EGYPTIAN FEMALE

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ABSTRACT

Several studies have reported the association of the SNP rs4077582 in the CYP11 gene with hyperandrogenism, which is one of the clinical manifestations of polycystic ovary syndrome (PCOS). These studies suggest that SNP rs4077582 may be involved in the etiopathogenisis of PCOS. To investigate whether the CYP11 gene SNP rs4077582 polymorphism is associated with the susceptibility to PCOS, we designed a case-controlled association study including 104 individuals. A case-controlled association study including 106 individuals (53 PCOS patients and 53 controls) was performed to assess the association of SNP rs4077582 with PCOS. Genotyping of SNP rs4077582 was conducted by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method that was performed on genomic DNA isolated from blood leucocytes. Results were analyzed in respect to clinical test results. The genotypic distributions of rs4077582 (CC, CT, TT) in the CYP11 gene in women with PCOS (CC, 29 (55.8%); CT, 20 (33.5%); and TT, 3 (5.8%). respectively) were significantly different from that in controls (CC, 44 (84.6%); CT, 6 (11.6%); and TT, 2 (3.8%) respectively) (P = 0.001). The allele frequencies in the PCOs women were: C, 78 (75%); and T, 26 (25%). This also was significantly different from the distribution in non-PCOs women: C, 94 (90.4%); and T, 10 (9.6%) (Table 2). In addition, **rs4077582** C > T showed a association with PCOs by logistic regression analysis controlling for confounding factors. Our data suggest that SNP **rs4077582** in the CYP11 gene is associated with susceptibility to PCOS.

Key words: CYP11A1, polycystic ovary, Egyptian

INTRODUCTION

Female infertility refers to infertility in female humans. It affects an estimated 48 million women with the highest prevalence of infertility affecting people in South Asia, Africa, North Africa/Middle East, Central/Eastern Europe and Central Asia. Infertility is caused by many sources, including nutrition, diseases, and other malformations of the uterus(Mascarenhas, Flaxman et al. 2012).

Causes and factors

According to the American Society for Reproductive Medicine (ASRM), Age, Smoking, Sexually Transmitted Infections, and Being Overweight or Underweight can all affect fertility. In broad sense, acquired factors practically include any factor that is not based on a genetic mutation, including any intrauterine exposure to toxins during fetal development, which may present as infertility many years later as an adult.

Tobacco smoking

Tobacco smoking is harmful to the ovaries, and the degree of damage is dependent upon the amount and length of time a woman smokes or is exposed to a smoke-filled environment. Nicotine and other harmful chemicals in cigarettes interfere with the body's ability to create estrogen, a hormone that regulates folliculogenesis and ovulation.

Sexually transmitted infections

Sexually transmitted infections are a leading cause of infertility. They often display few, if any visible symptoms, with the risk of failing to seek proper treatment in time to prevent decreased fertility(ASRM 2009).

Body weight and eating disorders

Twelve percent of all infertility cases are a result of a woman either being underweight or overweight. Fat cells produce estrogen, in addition to the primary sex organs. Too much body fat causes production of too much estrogen and the body begins to react as if it is on birth control, limiting the odds of getting pregnant. Too little body fat

causes insufficient production of estrogen and disruption of the menstrual cycle.

Chemotherapy

Chemotherapies with high risk of infertility include procarbazine and other alkylating drugs such as cyclophosphamide, ifosfamide, busulfan, melphalan, chlorambucil and chlormethine. Drugs with medium risk include doxorubicin and platinum analogs such as cisplatin and carboplatin. Female infertility by chemotherapy appears to be secondary to premature ovarian failure by loss of primordial follicles. Antral follicle count decreases after three series of chemotherapy(Mikkel Rosendahl, Claus Yding Andersen et al. 2010).

Other acquired factors

Adhesions secondary to surgery in the peritoneal cavity is the leading cause of acquired infertility(Herve´ Fernandez1 and Benoit Resch6 2013). There are other causes for female infertility like Diabetes mellitus (Simone L. Broer1 and Fauser1 2014), Coeliac disease (CD)(Chiara Tersigni1, Andrea Fattorossi1 et al. 2014) and Genetic factors(D.Bodri1 2006). There are many genes wherein mutation causes female infertility, which is believed to be genetic but where no single gene has been found to be responsible. Finally, an unknown number of genetic mutations cause a state of subfertility, which in addition to other factors such as environmental ones may manifest as frank infertility(D.Bodri1 2006). Some of these gene or chromosome abnormalities cause intersexed conditions, such as causes PCO Syndrome which is the common endocrinal disorder of infertility(2010* 2011).

In our studies we focused on endocrinological cause's specifically polycystic ovary syndrome (PCOs).

Polycystic ovary syndrome (PCOS) is a condition which can affect a woman's menstrual cycle, fertility, hormones and aspects of her appearance. It can also affect long-term health. Polycystic ovaries are slightly larger than normal ovaries and have twice the number of follicles (small cysts)(Gynaecologists 2009). The cause of PCOS is not yet known, it is run in families. If any of your relatives (mother, aunts, sisters) are affected with PCOS, your own risk of developing PCOS may be increased this character strongly suggests that PCOs associated with specific gene or genes mutation(s) (Gynaecologists 2009).

PCOs Diagnosis

PCOs diagnosed by positive clinical symptoms and sign, which must be confirmed by radiological & biochemical lab. Investigations for three main parameter, FSH, LH & Testosterone, other hormones are involved such as prolactin & insulin as well.

PCOs and genetic association

Thirty seven genes may involve in PCOs, this genes arranged according to effect on: Steroid formation as CYP19, CYP11A. Gonadotropins as FS, ACTR.FSHR, Obesity as D7S1875, D1S198. and Insulin action as IGF1R, IGF1(MARGRIT URBANEK* and ROBERT J. NORMANi 1999). Due to our dependency on FSH, LH and Testosterone in diagnosis we focusing on steroid and gonadotropins associated genes to study polymorphism in some of these genes as , CYP19, &FS and focusing specially on CYP11A1 in Egyptian females.

MATERIAL AND METHODS

Subjects

A sum of 106 females between (18-45 years old) with no medical treatment taken at least three months before blood sample collected .the patient consented restrictedly according E.M.S.(Egyptian Medical Syndicate) instructions& ethics.

53 PCOS patients and 53 non-PCOS controls ladies were included. Every one of these controls had ordinary menstrual cycles (32 days) and typical body mass record (BMI), barring hyperandrogenism hirsutism, diabetes mellitus, and so forth. The participated were gathered in Department of Obstetrics and Gynecology Koom Hamada hospital-Bhera Governate-Egypt. 53 PCOs ladies were included had explored for hormonal and BMI in the wake of taking history and clinical examination. The partook were gathered in Department of Obstetrics and Gynecology Tanta University.

PCOS diagnostic criteria and hormone measurements

PCOS were analyzed by the overhauled 2003 agreement on demonstrative criteria and long haul health dangers identified with polycystic ovary disorder Simply, no less than two of taking after three criteria was required: Clinical and/or

biochemical indication of hyperandrogenism, oligomenorrhea or anovulation, and polycystic ovarian morphology on ultrasound .Meanwhile, different illnesses which could bring about hyperandrogenism ought to be rejected, for example, intrinsic adrenal hyperplasia hypothyroidism, androgen-emitting tumors, Cushing's disorder, and so on. Body mass index was figured as body weight in kilograms partitioned by square of stature in meters. Age was acquired through request. None of the members had gotten or been accepting hormone medications or oral contraceptives for the past 3 months. Fringe blood of all members who had period was gathered amid the third to the fifth day of the menstrual cycle and whenever for the individuals who had amenorrhea. All blood tests were collected between 8 AM and 9 AM after a 12h overnight quick. Regenerative hormone levels were measured by RIA (Tanta University Biochemical Lab.), including downright (testosterone (T), follicle-empowering hormone (FSH) luteinizing hormone (LH), and prolactin (PL).

Polymorphism genotyping analysis.

Genomic DNA was isolated from leukocytes by using G-spin™ Total DNA Extraction Mini Kit.(Intron Biotechnology,Korea.) then stored at 20 C.

Polymorphism genotyping analysis CYP11A1 gene

Polymerase chain response (PCR) utilizing particular groundworks to genotyping the succession (5GCC AGT CAG ACA AGG GCACAG GA-3 forward)& (5GTG GCC GAC TAT GTAAAC CAG-3 reverse) for rs4077582. An aggregate volume of 2ul of genomic DNA ,10 pmol of each primer(Biolegio BV-Motherland) , (forward:molecular weight 7117gr\mol,100uM Solution dissolve in 147.90 ul water.total bases 23,CG% 61. also, reverse:molecular weight 6455gr\mol, 1oouM broke up in 196.88ul water,,total bases 21 and CG%52.),6ul of refined water,10ul of DNA Taq polymerase (expert blend Intron-Korea) was utilized for the PCR response with aggregate volume 20 ul. The PCR was performed in MultiGene Gradient Thermal Cycler (USA) as follows: 36cycles including 1 min of denaturation at 94^{o}C, 1 min of annealing at 58^{o}C, and 55 min of extension at 72^{o}C. An initial step of 2 min at 96 ^{o}C denaturation and a final extension of 15 min at 72^{o}C were added.. Afterwards, electrophoresis was used to separate DNA fragments on a 3 % agarose gel stained with ethidium bromide.

Statistical analysis

Clinical variables and hormone levels were all reported as mean ± SD. Fisher's Exact Test was used in genotypic distribution analysis in our case–control study. Clinical variables and serum hormone levels were all compared by one-way analysis of variance (ANOVA), Turkey-test was used for further analysis of the differences among the three genotypes. Genotype frequencies were checked for Hardy–Weinberg equilibrium in both PCOS and control groups. P\0.05 was considered statistically significant. All the analyses were performed using SPSS Statistics Version 17.0.

RESULTS AND DISCUSSION

Characteristics defining PCOS and control groups Clinical variables and hormone levels of PCOS patients and controls were shown in (Table 1&2), which is consistent with 2003 Rotterdam PCOS diagnose criteria. According to our molecular data obtained from PCR product of (CYP11a1) after gel electrophoresis reveal that genotypic distributions of the SNP rs4077582 in PCOS was significantly different from the controls (figure 1&2).

Clinical characteristics of obese women

The clinical characteristics of the PCOs women and controls are presented in Figure 1. We can see there is a significance correlation between the BMI and the Age more than the control, good significance between PCOs and LH level. On the contrary the FSH measurements of the PCOs women did not gave significant results.

Frequencies of alleles and genotypes

Genotype frequencies in all groups were in accordance with the Hardy- Weinberg equilibrium. The distribution of **rs4077582** genotype in the PCOs women was as follows; CC, 29 (55.8%); CT, 20 (33.5%); and TT, 3 (5.8%). This was significantly different from the distribution in the non-PCO women: CC, 44 (84.6%); CT, 6 (11.6%); and TT, 2 (3.8%). The allele frequencies in the PCOs women were: C, 78 (75%); and T, 26 (25%). This also was significantly different from the distribution in non-PCOs women: C, 94 (90.4%); and T, 10 (9.6%) (Table 2). In addition, **rs4077582** C > T showed a association with PCOs by logistic regression analysis controlling for confounding factors.

Biochemical lab statistical analysis

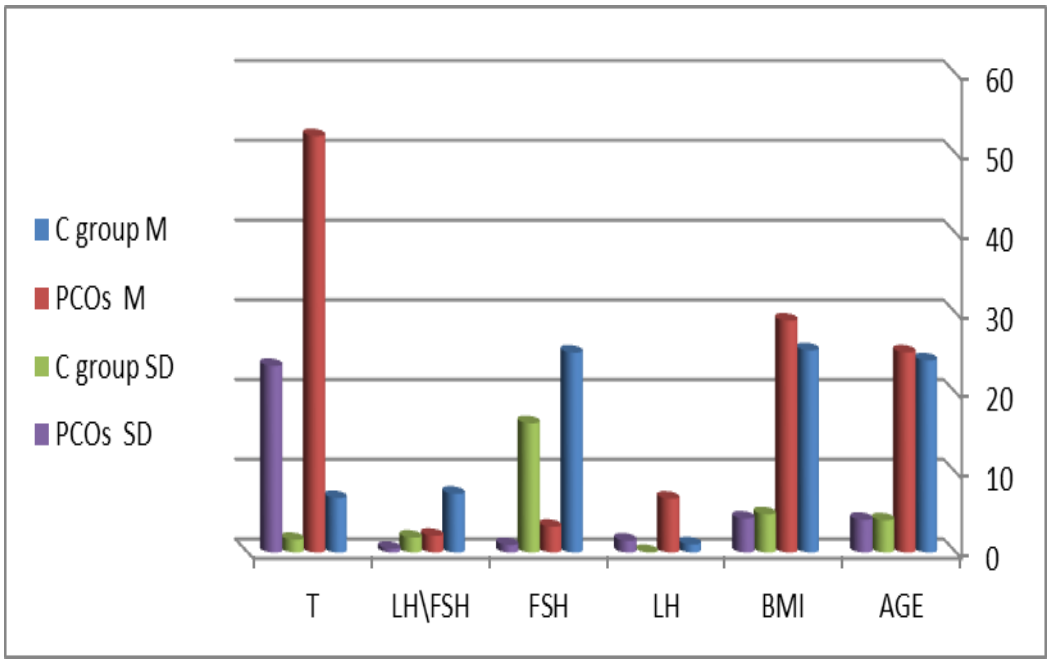


Fig (1). Show The Clinical variables Characteristics differences between PCOS patient's and control groups.

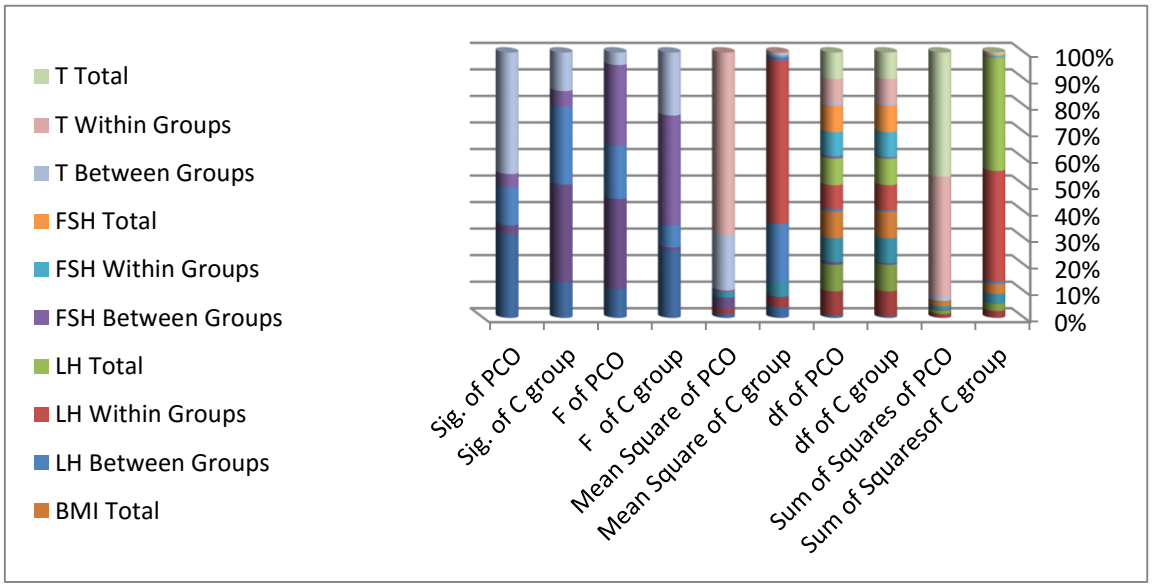
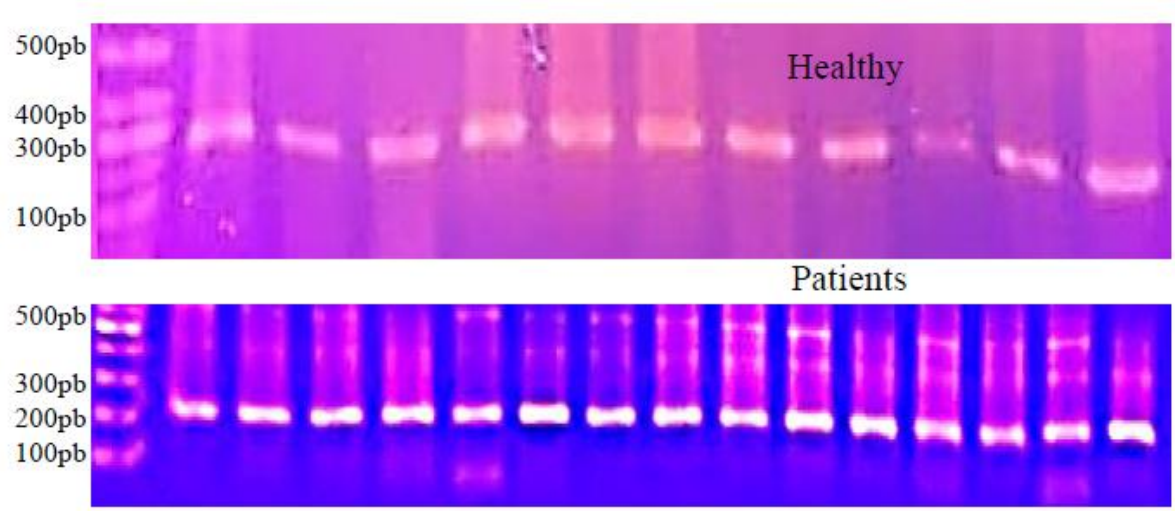


Fig (2). Show The Clinical variables Characteristics differences between PCOS patient's and control groups

In this study, rs4077582 was observed to be connected with the pathogenesis of PCOS, indicating that rs4077582 might influence the P450scc compound action and subsequently impact, androgen generation. This conclusion was further validated by the fluctuating testosterone levels inside of the three genotypes of rs4077582. Rs4077582 is situated at the 50-upstream regulation locale. It is sure that this SNP, and also the (TTTTA)n succession, is arranged in the promoter zone. In our study, the LH levels were fundamentally different among the three genotypes of rs4077582, with a higher LH level in the CT genotype, as contrasted and the CC and TT genotypes. LH fortifies the theca cell segment of the follicle and impels creation of



(Fig. 1) Electrophoresis of DNA (CYP11a1) rs4077582 fragments before and after digestion with restriction. The Electrophoresis of DNA fragments revealed a single 281-bp band stood for homozygote. Bands of 210- and 70- bp represented heterozygote.

	Group Ⅰ (Control) 53		Group Ⅱ (PCO) 53		
	n	**(%)**	**n**	**(%)**	***P****
Genotype frequencies					
CC	44	84.6	29	55.8	$x^2 = 0.216$
CT	6	11.6	20	38.5	*P* = 0.012
TT	2	3.8	3	5.8	
Allelic frequencies					
C	94	90.4	78	75	
T	10	9.6	26	25	

Table (2): The frequency distributions combined with previous data of the rs4077582 in women with PCOS and controls

androgens LH drives the declaration of CYP11A1 in the theca cells of creating follicles. Gonadotrophins, including LH, are the essential controllers of CYP11A1 expression and enhance CYP11A1 promoter action in theca, granulosa, and luteal cells [15, 16]. Gonadotrophins incite granulose and luteal cell steroidogenesis by acting through the cAMP second detachment flagging framework. CYP11A1 expression is improved by the LH surge and safeguarded at an abnormal state in luteal cells(PANCHARATNAM JEYASURIA 2004) . Moreover, development components perform a key part in gonadotrophin's simulative impact on the CYP11A1 expression. A few studies reported that insulin-like development component (IGF-2) and epidermal development element (EGF) might expand LH and FSH incitement(Haouzi, 1 et al. 2012)&(Nelson-DeGrave VL1 2003) . Insulin-like development component 1 (IGF-1) upgrades the impacts of gonadotrophin in a complex way which

might influence SNPs other than CYP11A1 (PANCHARATNAM JEYASURIA 2004). Moreover, estradiol, progesterone, development hormone, and activin A can advance CYP11A1 expression (MARGRIT URBANEK* 2006). A few studies on the regulation of CYP11A1 promoter recommend that Steroidogenic element 1 (SF-1) and liver receptor homologue 1 (LRH-1) can drive human CYP11A1 promoter in granulosa cells (Zhang 2012). Particular knockout of SF-1 could cause a cataclysmic loss of P450scc (MARGRIT URBANEK* and ROBERT J. NORMANi 1999). The system by which gonadotrophins associate with CYP11A1 stays vague in this way. Thus people with various genotypes of rs4077582 might demonstrate particular reaction patterns to LH boost in light of fluctuating LH levels in various genotypes which affects downstream steroid hormone levels. Not surprisingly, testosterone of the heterozygous CT genotype demonstrated a fundamentally more elevated amount contrasted with that of TT genotype, likely decidedly controlled by LH. While in PCOS patients, no distinction was saw in the LH levels among the three genotypes of rs4077582, most likely because of the strange inward emission of LH in PCOS patients. Strikingly, control people with the CT heterozygous genotype of rs4077582 demonstrated an altogether higher testosterone level and LH/FSH proportion contrasted with that of CC and TT genotypes, however no distinction was found in testosterone levels between the CC and CT genotype. These bits of evidence showed that typical control ladies with the CT genotype of rs4077582 were more liable to create PCOS, which was upheld by the way that PCOS patients had a greater bit of people with the CT genotype when contrasted with controls. The framework by which gonadotrophins partner with CYP11A1 stays ambiguous along these lines. Thus individuals with different genotypes of rs4077582 may exhibit specific response patterns to LH support in light of fluctuating LH levels in different genotypes which affects downstream steroid hormone levels. As anyone might expect, testosterone of the heterozygous CT genotype showed an on a very basic level more hoisted sum appeared differently in relation to that of TT genotype, likely firmly controlled by LH. While in PCOS patients, no refinement was found in the LH levels among the three genotypes of rs4077582, in all probability as a result of the weird internal emanation of LH in PCOS patients. Strikingly, control individuals with the CT heterozygous genotype of rs4077582 showed an out and out higher testosterone level and LH/FSH extent stood out from that of CC and TT genotypes, however no refinement was found in testosterone levels between the CC and CT genotype. These bits of evidence demonstrated that run of the mill control women with the CT genotype of rs4077582 were more obligated to make PCOS, which was maintained by the way that PCOS patients had a greater piece of individuals with the CT genotype when diverged from controls. To our best knowledge, this is one of the first studies demonstrating the association between SNPs in CYP11A1 and PCOS.All things considered, a few studies have reported the relationship of a microsatellite polymorphism (TTTTA)n in the promoter of CYP11A1 with PCOS. Gharani showed its solid relationship with the serum testosterone level. While in the study on Spanish women, no affiliation was accounted for with hirsutism or hyperandrogenism . After a huge scale investigation, McCarthy found that the (TTTTA)n polymorphism was associated with androgenrelated genotypes . Concentrates on Chinese ladies have also been led, however no cognizant conclusion is come to. Without a sufficiently vast example size, Tan and Chen exhibited no connection of this polymorphism with PCOS (Haouzi, 1 et al. 2012). In actuality, our past study proposed a specific part the (TTTTA)6 variant played in the pathogenesis of Chinese ladies with PCOS (Zhang 2012). Yet some different studies gave careful consideration to CYP11A1 expression and insulin levels to examine the relationship in the middle of CYP11A1 and PCOS. Mamluk and Sekar illustrated that insulin could upgrade the outflow of CYP11A1 in the luteinized granulosa cells (Nelson-DeGrave VL1 2003). As of late critical relationship between's uncoupling protein-2, which contrarily regulates affectability to insulin, and CYP11A1 expression was found in PCOS patients . In outline, SNP rs4077582 in CYP11A1 is unequivocally connected with weakness to PCOS and might adjust testosterone levels by the regulation of LH in various genotypes. However, PCOS can't be connected with a solitary variable of

this quality, more research on predisposing reasons for PCOS and the capacity of CYP11A1 quality is required later on.

In summary, SNP rs4077582 in CYP11A1 is emphatically connected with powerlessness to PCOS and might modify testosterone levels by the regulation of LH hormone. more research on predisposing reasons for PCOS and the capacity of CYP11A1 quality is required in the future.

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