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Short Communication

Polymorphisms of transcription factor-7-like 2 (*TCF7L2*) gene in Tunisian women with polycystic ovary syndrome (PCOS)

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ABSTRACT

Background and aims: Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects women in their child-bearing age, and is often associated with insulin resistance and type 2 diabetes (T2DM). Given the overlap between PCOS and T2DM, we investigated the association of transcription factor-7-like 2 (*TCF7L2*) variants rs4506565, rs7903146, rs12243326, and rs12255372 with the susceptibility to PCOS.
Subjects and methods: Study subjects comprised 119 Tunisian women with PCOS (mean age 29.8 ± 4.7 years), and 150 control women (mean age 30.6 ± 5.9 years). *TCF7L2* genotyping was done by the allelic discrimination/real-time PCR method.
Results: Minor allele frequencies (MAFs) of rs4506565 ($P = 0.61$), rs7903146 ($P = 0.68$), rs12243326 ($P = 0.56$), and rs12255372 ($P = 0.60$) were comparable between PCOS cases and control subjects. As the four tested *TCF7L2* variants were in linkage disequilibrium, 4-locus (rs4506565, rs7903146, rs12243326, rs12255372) haplotype analysis demonstrated that haplotype 2111 was initially negatively associated with PCOS [$P = 0.035$; OR (95% CI) = 0.13 (0.02–0.85)], which was later lost upon correcting for multiple comparisons [$Pc = 0.248$].
Conclusion: Our data suggest that there is weak or no contribution of *TCF7L2* gene polymorphism to PCOS in Tunisian women. Further studies with larger samples are necessary to confirm this observation.
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1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy, affecting 6–10% of women in their reproductive age (Goodarzi et al., 2011a, 2011b; Wild et al., 2010). It is characterized by chronic anovulation, hyperandrogenism, and polycystic ovarian morphology on ultrasonography (Azziz et al., 2009; Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). PCOS is also associated with obesity, insulin resistance (Galluzzo et al., 2008), pancreatic β-cell dysfunction, and increased risk for metabolic syndrome and type 2 diabetes mellitus (T2DM) (Teede et al., 2010). PCOS is multifactorial in nature, and environmental and genetic risk factors were reported to contribute to its development (Roldán et al., 2004). PCOS shares with T2DM common features, thus suggesting genetic contribution to the susceptibility to both disorders (Franks, 1995). T2DM associated loci, including *FTO* (fat mass and obesity associated; Ewens et al., 2011; Wojciechowski et al., 2012), *MCR4* (melacocortin-4; Ewens et al., 2011), *CAPN10* (calpain-10; González et al., 2002; Márquez et al., 2008), PPARG (peroxisome proliferator-activated receptor gamma; Tang et al., 2012), *INSR* (insulin receptor; Goodarzi et al., 2011a, 2011b; Xu et al., 2011) and *TCF7L2* (transcription factor-7-like 2) (Christopoulos et al., 2008; Xu et al., 2010) were also reported to contribute to PCOS pathogenesis.

TCF7L2 gene, also known as *TCF4*, is located on chromosome 10q25.3 (Duval et al., 2000), and encodes a high-mobility group box-containing transcription factor, implicated in blood glucose homeostasis (Helgason et al., 2007). *TCF7L2* acts predominantly by regulating pro-glucagon expression via Wnt signaling pathway by repressing the proglucagon gene in enteroendocrine cells (Yi et al., 2005), and is associated with β-cell dysfunction and reduced insulin secretion (Florez et al., 2006). A relationship between *TCF7L2* variants and PCOS was suggested by some, but not all studies, exemplified by the association of rs7903146 *TCF7L2* variant with PCOS among Greek (Christopoulos et al., 2008), but not Chinese (Xu et al., 2010), or Korean (Kim et al., 2012) PCOS women.

The aim of this study was to investigate the association between rs4506565, rs7903146, rs12243326, and rs12255372 *TCF7L2* gene polymorphisms and PCOS among 119 Tunisian PCOS patients and 150 control subjects. To the best of our knowledge, this is the first study to

Abbreviations: BMI, body-mass index; HOMA-IR, homeostasis model assessment for insulin resistance; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; PCOS, polycystic ovary syndrome; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes; *TCF7L2*, transcription factor-7-like 2.

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examine the association of the four *TCF7L2* variants with PCOS, and the first to identify specific *TCF7L2* haplotypes in a North African community (Tunisia).

2. Subjects and methods

2.1. Subjects

Between January 2011 and October 2011, 134 consecutively-recruited women with confirmed PCOS diagnosis (mean age: 30.1 ± 4.1 years) were recruited from the outpatient Obstetrics and Gynecology Service, CHU Tahar Sfar (Mahdia), and CHU Farhat Hached (Sousse) in Central Tunisia. PCOS diagnosis was based on the 2003 Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004), in which PCOS diagnosis was established when two of the following three conditions were met: anovulation, hyperandrogenism, and the presence of polycystic ovary on ultrasound examination. Exclusion criteria were consistent with PCOS definition, and included other causes of hyperandrogenism, such as androgen-secreting tumors and nonclassical adrenal hyperplasia, hyperprolactinemia, Cushing's syndrome, diabetes, hypertension (blood pressure > 140/90 mm Hg), known cardiovascular disease, active thyroid disease, and renal disease. Refusal to participate in the study was low among cases (n = 15), and absent from control women, and was related to lack of sufficient data (n = 4), to the presence of at least one of the exclusion criteria (n = 8), and to sampling problems (n = 3). This left a total of 119 women eligible for investigation.

Control group consisted of 150 healthy women (mean age: 31.8 ± 5.7 years), with regular menstrual cycles and no evidence of hirsutism, acne, alopecia, or endocrinopathies. None of the controls was on hormonal therapy (including oral contraceptives) for the previous three months or longer, and none was related to PCOS cases or control women, or was on medication known to affect carbohydrate metabolism or endocrine parameters for at least three months before entering the study. Demographic data and history of hypertension, diabetes, and hypercholesterolemia were recorded for all subjects. Obesity was defined as body-mass index (BMI) ≥ 30 kg/m^2. The study protocol was approved by the local ethics committee, and written informed consent was obtained from all subjects.

2.2. Biochemical analysis

Blood samples were collected after an overnight (>12 h) fast for measurement of glucose, insulin, triglycerides, total cholesterol, and HDL levels. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as per: HOMA-IR = [insulin (μIU/ml) × glucose (mmol/l)] / 22.5. Follicle stimulating hormone, luteinizing hormone, prolactin, testosterone, progesterone, 17α-hydroxyprogesterone, and thyroid stimulating hormone were determined using immunofluorometric assay or radioimmunoassay.

2.3. SNP genotyping

Total genomic DNA was isolated from peripheral blood lymphocytes of study subjects by the salting-out method. We selected *TCF7L2* rs4506565, rs7903146, rs12243326, and rs12255372 single nucleotide polymorphisms (SNPs), in view of their relatively high frequency in Caucasians, and their reported association with T2DM. The four SNPs were genotyped by the allelic discrimination method on StepOne real-time PCR system (Applied Biosystems; Foster City, CA); using commercially available primers obtained from the Assay-on-Demand system with well-defined genotype clusters. Replicate quality control samples and negative controls were independently and blindly genotyped to ensure the reproducibility of the genotyping procedure; concordance was >99%. Genotype frequencies of the four SNPs were consistent with Hardy–Weinberg equilibrium (Table 2), and the minor allele frequencies (MAFs) obtained were comparable to those in the HapMap CEU sample.

2.4. Statistical analysis

Data were expressed as mean ± SD (continuous variables) or as percent of total (categorical variables), and intergroup significance was assessed by Student's *t*-test (continuous variables), and χ^2 test (categorical variables), using IBM SPSS Statistics version 19. Allele frequencies were calculated by gene-counting method; each SNP was tested for Hardy–Weinberg equilibrium (HWE), using HW Calculator (http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls). Pairwise linkage disequilibrium (LD) values were calculated with HaploView 4.2, which also computed the frequency of the common 4-locus haplotypes. Multiple comparison correction was performed by the Bonferroni's method, using *Simple Interactive Statistical Analysis* (SISA) online calculator (www.quantitativeskills.com/sisa/calculations/bonhlp.htm). Null hypothesis was rejected at $P < 0.05$.

3. Results

3.1. Study subjects

The clinical features of women enrolled in the study are summarized in Table 1. While the mean age of patients was similar to that of controls ($P = 0.209$), BMI, serum cholesterol, triglycerides, LDL, HDL, glucose, and total testosterone were higher in PCOS women than in control women. Accordingly, age, BMI, and lipid profile (LDL, total cholesterol, triglycerides) were selected as the covariates that were controlled for in subsequent analysis.

3.2. Association studies

The association between *TCF7L2* SNPs and PCOS in case–control subjects is summarized Table 2. rs4506565 ($P = 0.232$), rs7903146 ($P = 0.523$), rs12243326 ($P = 0.300$), and rs12255372 ($P = 0.139$) were in HWE among control women. Minor allele frequencies (MAFs) of rs4506565 ($P = 0.612$), rs7903146 ($P = 0.680$), rs12243326 ($P = 0.561$), and rs12255372 ($P = 0.600$) were comparable between PCOS cases and control subjects. Table 3 summarizes the results of association between rs4506565, rs7903146, rs12243326, and rs12255372 *TCF7L2* genotypes and PCOS, under additive genetic model (as it is the conservative model), after adjusting for the covariates BMI, age, and lipid profile (LDL, total cholesterol, triglycerides). Of the four variants tested, we showed no significant association with PCOS: rs4506565 ($P = 0.49$), rs7903146 ($P = 0.29$), rs12243326 ($P = 0.61$), and rs12255372 ($P = 0.29$).

Table 1
Demographics and clinical characteristics of the study subjects.

	Cases[a]	Controls[a]	*P*[b]
Age at inclusion in study[c]	29.8 ± 4.7	30.6 ± 5.9	0.209
Fasting glucose (mmol/l)	7.6 ± 2.1	5.6 ± 3.4	0.008
Insulin (μU/ml)	16.4 ± 11.8	7.1 ± 3.0	<0.001
HOMA-IR	5.5 ± 4.6	2.2 ± 2.0	0.028
Body-mass index (kg/m^2)[c]	28.4 ± 7.1	24.5 ± 3.8	<0.001
FSH levels (mIU/ml)	5.0 ± 1.9	4.0 ± 2.2	0.005
LH levels (mIU/ml)	6.2 ± 4.1	4.1 ± 3.0	0.008
Estradiol levels (pg/ml)	3309.2 ± 720.5	339.4 ± 246.9	<0.001
Testosterone levels (ng/ml)	3.72 ± 3.62	0.98 ± 0.71	<0.001
HDL	1.42 ± 0.4	1.35 ± 0.5	0.537
LDL	3.02 ± 0.9	1.92 ± 0.7	<0.001
Triglycerides	1.68 ± 0.7	0.97 ± 0.4	<0.001
Cholesterol	5.98 ± 1.5	3.83 ± 0.9	<0.001

[a] A total of 119 PCOS cases and 150 control subjects were included.
[b] Student's *t*-test for continuous variables; Pearson's chi square test for categorical variables.
[c] Mean ± SD.

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