Gene 647 (2018) 79–84

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Differential association of *DENND1A* genetic variants with polycystic ovary syndrome in Tunisian but not Bahraini Arab women☆

Meriem Dallel[a], Sameh Sarray[b,c], Zeineb Douma[a], Feten Hachani[d], Abrar K. Al-Ansari[c], Dhafer B. Letaifa[d,e], Touhami Mahjoub[a], Wassim Y. Almawi[b,*]

[a] Laboratory of Human Genome and Multifactorial Diseases (LR12ES07), Faculty of Pharmacy of Monastir, University of Monastir, Tunisia
[b] Faculty of Sciences, El Manar University, Tunis, Tunisia
[c] College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Bahrain
[d] CHU Farhat Hached, Sousse, Tunisia
[e] Faculty of Medicine, Sousse, Tunisia

ARTICLE INFO

Keywords:
DENND1A
Genotyping
Haplotype
Polycystic ovary syndrome
Single nucleotide polymorphisms

ABSTRACT

Background and aim: Polycystic ovary syndrome (PCOS) is a common endocrine disorder, and results from interaction between modifiable and non-modifiable factors, including genetic predisposition. Previous genome-wide association studies and meta-analysis identified *DENND1A* as PCOS susceptibility locus in some, but not all populations. We investigated whether the association of *DENND1A* gene variants with PCOS was similar between Tunisian and Bahraini Arab women.

Subjects and methods: This was retrospective case-control study. Study subjects comprised 320 women with PCOS, and 446 age-and ethnically-matched control women. Genotyping of *DENND1A* rs10818854, rs2479106, and rs10986105 variants was done by real-time PCR.

Results: Minor allele frequency of rs10818854 and rs10986105 *DENND1A* variants were significantly higher among women with PCOS. Setting homozygous wild-type genotype carrier as reference, rs10818854 and rs10986105 were associated with increased risk of PCOS, which persisted after controlling for key covariates, while reduced PCOS risk was seen with only rs2479106 under the additive model. This assigned PCOS susceptibility and protective nature to these genotypes, respectively. Both rs10818854 and rs10986105 were positively associated with HOMA-IR and AMH in women with PCOS. Haploview analysis revealed limited linkage disequilibrium between the tested *DENND1A* variants. Extensive diversity in haplotypes assignment was seen, with most haplotypes (99.5%) captured by 5 haplotypes. Taking GAT haplotype as reference, AAG, and GAG haplotypes were positively, while GAT haplotype was negatively associated with PCOS.

Conclusion: The association of *DENND1A* rs10818854 and rs10986105 variants with PCOS in Tunisian but not Bahraini women confirms the dependence of this association on the ethnic/racial origin of study subjects.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common, but complex endocrine disorder in women of reproductive age. With 6.5–15% prevalence worldwide, PCOS ranks as the most common cause of infertility (Azziz et al., 2004; Dumesic et al., 2015; Goodarzi and Azziz, 2006). PCOS is characterized by menstrual irregularity manifested as oligo-ovulation or anovulation, biochemical and/or clinical hyperandrogenism, and polycystic ovarian (PCO) morphology (Azziz et al., 2004; Dumesic et al., 2015; Legro et al., 1998). Several metabolic aberrations accompany PCOS, including obesity, insulin resistance and altered glucose homeostasis, dyslipidemia, and higher prevalence of metabolic syndrome features. This translates into increased risk of type 2 diabetes mellitus (T2DM), and cardiovascular disease (CVD) (Azziz et al., 2004; Diamanti-Kandarakis and Dunaif, 2012; Goodarzi and Azziz, 2006; Wild et al., 2010). PCOS has a heterogeneous phenotype, which varies according to race and ethnicity, and is exacerbated by life style factors, most notably obesity (Dumesic et al., 2015; Legro et al.,

Abbreviations: AMH, anti-Müllerian hormone; BMI, body-mass index; CVD, cardiovascular disease; DENND1A, differentially expressed in normal and neoplastic cells domain-containing protein 1A; GWAS, genome-wide association studies; HWE, Hardy–Weinberg equilibrium; HOMA-IR, homeostasis model assessment-insulin resistance; MAF, minor allele frequencies; PCOS, polycystic ovary syndrome; T2DM, type 2 diabetes mellitus

☆ Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

* Corresponding author at: Faculty of Sciences Tunis, University of Tunis El Manar, University Campus, 2092 Manar II, Tunis, Tunisia.
E-mail address: wassim.almawi@outlook.com (W.Y. Almawi).

https://doi.org/10.1016/j.gene.2018.01.028
Received 8 November 2017; Accepted 6 January 2018
Available online 08 January 2018
0378-1119/

1998). Despite the impact of PCOS on women's wellbeing, its underlying pathogenesis remains poorly understood.

PCOS is a complex polygenic disorder, and its pathogenesis is influenced by modifiable/environmental, and non-modifiable factors, including genetic predisposition (Azziz et al., 2004; Chen et al., 2011; Legro et al., 1998; Louwers et al., 2013). The latter was highlighted by populations-based familial aggregation and twin studies of PCOS (Dumesic et al., 2015; Goodarzi and Azziz, 2006; Lerchbaum et al., 2014; Vink et al., 2005), and genome-wide association studies (GWAS), which identified several PCOS candidate loci (Chen et al., 2011; Cui et al., 2013), which appear to be racially-determined (Wang and Alvero, 2013; Zhao and Qiao, 2013). The latter included gene variants in gonadotropin receptors, thyroid adenoma-associated protein (THADA), insulin receptor (INSR), and differentially expressed in normal and neoplastic cells domain-containing protein 1A (DENND1A) (Chen et al., 2011; Day et al., 2015; Shi et al., 2012).

DENND1A is a member of the connecdenn family of 18 human genes, and encodes a protein DENN/MADD domain containing 1A, or DENND1A. This protein is a component of clathrin-mediated endocytosis machinery (Marat and McPherson, 2010), and functions as guanine nucleotide exchange factor for Rab35 in regulating endosomal membrane trafficking (Allaire et al., 2010; Marat et al., 2011). *DENND1A* gene is localized on chromosome 9 (9q22.32), and alternative splicing yields two principal transcripts DENND1A variant 1 and DENND1A variant 2, which is differentially expressed in theca cells of women with PCOS (McAllister et al., 2014). DENND1A gene is polymorphic, and several *DENND1A* gene variants were reported, including rs10818854, rs2479106, and rs10986105, which were reportedly associated with PCOS in Han Chinese women (Chen et al., 2011).

Bahraini (Gammoh et al., 2015), Danish (Eriksen et al., 2012), USA-Caucasian (Goodarzi et al., 2012), and multi-European (Welt et al., 2012) replication studies yielded variable results on the association of *DENND1A* variants with PCOS, suggesting contribution of ethnicity in this association. The present study explores the association of the common *DENND1A*gene variants rs10818854, rs2479106, and rs10986105 with PCOS in Tunisian Arab population, the first to examine this association in a North African population.

2. Material and methods

2.1. Study subjects

This case-controlled study was performed at outpatient OB/GYN and endocrinology clinics of Farhat Hached Hospital of Sousse, Tunisia, and involved 320 women with PCOS, and 446 age-and ethnically-matched control women, who were recruited into the study in the period January 2013–December 2015 (Table 1). The diagnosis of PCOS was based on the 2003 Rotterdam Criteria (Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop Group, 2004), according to which the diagnosis of PCOS was confirmed if at least two of the three criteria were fulfilled, oligo-ovulation or anovulation, presence of polycystic ovaries on ultrasound, and clinical and/or biochemical signs of hyperandrogenism.

Exclusion criteria included hyperprolactinemia, active thyroid disease and hyperandrogenism of unrelated etiologies, such as congenital adrenal hyperplasia, and Cushing's syndrome. Additional exclusion criteria included extremes of body mass index (BMI; < 18 or > 50 kg/m^2), current illness, medications likely to affect carbohydrate metabolism or endocrine parameters for at least 3 months before entering the study. Control population comprised eumenorrheic university students and employees, and healthy female volunteers from the community. The androgen levels of control women were within reference range (0.4–3.5 nmol/L), and were studied in the follicular phase of their menstrual cycle.

Demographic data, and history of hypertension, diabetes and hypercholesterolemia were taken from all participants. All patients underwent transvaginal ultrasound scanning for PCOS and modified Ferriman-Gallwey scoring for the identification of hirsutism. Subjects were deemed hirsute if the score was six or higher. Body-mass index (BMI) was calculated as per: weight (kg) / height (m^2). Study participants gave written informed consent before entering the study, which was approved by research and ethics committees of Farhat Hached Hospital of Sousse and University of Monastir.

Table 1
Baseline and endocrine parameters of women with PCOS and control women.

	Controls (n = 446)	Cases (n = 320)	P^a
Age (years)[b]	31.83 ± 6.0	30.85 ± 4.7	.019
BMI (kg/m^2)[b]	25.93 ± 5.4	29.22 ± 6.1	< .0001
Waist-Hip ratio[b]	0.89 ± 0.05	0.90 ± 0.08	.215
LH (IU/L)[c]	4.4 (1.1–22.6)	5.86 (0.2–19.7)	1.0×10^{-6}
FSH (IU/L)[c]	7.9 (2.17–24)	6.4 (2.06–14.6)	< .0001
LH/FSH[b]	0.60 ± 0.3	1.09 ± 0.7	< .001
Total testosterone (nmol/L)[c]	0.8 (0.28–5.28)	2.5 (0.2–32.6)	< .0001
Estradiol (nmol/L)[c]	50 (13.12–300)	48.8 (8–580)	.328
SHBG (nmol/L)[c]	41.96 (6.9–266.24)	55.8 (1.2–290.68)	.09
Prolactin (nmol/L)[c]	16.6 (0.2–450)	18.7 (0.6–680)	.022
Fasting glucose (mmol/L)[b]	4.13 ± 1.2	6.5 ± 2.6	< .0001
Insulin (mIU/L)[c]	7.42 (1.15–53)	10.85 (0.75–80.2)	.02
HOMA-IR[b]	2.67 ± 3.2	5.8 ± 7.2	2.9×10^{-4}
Triglycerides (mmol/L)[b]	1.33 ± 0.8	1.66 ± 1	.075
Cholesterol (mmol/L)[b]	4.42 ± 0.8	5.49 ± 1.5	< .0001
HDL (mmol/L)[b]	3.95 ± 16.5	1.27 ± 0.42	.345
LDL (mmol/L)[b]	2.65 ± 0.7	2.95 ± 0.9	.076
TSH (μIU/mL)[b]	2.22 ± 1.3	2.13 ± 1.34	.638
FT4	11.54 ± 3.4	13.59 ± 3.6	.25

[a] Student's *t*-test (variable with normal distribution), Mann–Whitney *U* test (variables that were not normally distributed).
[b] Mean ± SD.
[c] Median (range).

2.2. Biochemical analysis

Fasting blood sample was obtained during the early follicular phase (between 2 and 5 days of the menstrual cycle) for PCOS cases with regular menses and control women, or any day for PCOS cases with menstrual disturbances (oligoovulation, anovulation). Serum samples were assayed for follicular-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (T), estradiol (E_2), prolactin (PRL) and anti-Müllerian hormone (AMH) by chemiluminescence immunoassays, all with intra- and inter-assay coefficients of variation < 10%. Insulin and SHBG were measured by ELISA. Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR), calculated as per: fasting insulin (μIU/mL) × fasting plasma glucose (nmol/L)/22.5.

2.3. DENND1A genotyping

Total genomic DNA was isolated from peripheral venous blood samples of study subjects by the salting-out method. The tested DENND1A polymorphisms were selected in light of previous studies linking them with PCOS, and their frequency in Caucasians (minor allele frequencies; MAF > 5%). *DENND1A* genotyping was done using the allelic (VIC- and FAM-labelled) discrimination method. Assay-on-demand TaqMan assays were ordered from Applied Biosystems: C_32123858_20 (rs10818854), C_11834613_10 (rs2479106), and C_32123935_10 (rs10986105). The reaction was performed in 6 μL volume on StepOne Plus PCR system, per manufacturer's instructions (Applied Biosystems). Replicated blinded quality control samples were included to assess reproducibility of the genotyping procedure, concordance was > 99%. *DENND1A*genotype frequencies were consistent