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Replication of Association of *DENND1A* and *THADA* Variants with Polycystic Ovary Syndrome in European Cohorts

Mark O Goodarzi[1,2,3,4], **Michelle R Jones**[1], **Xiaohui Li**[3], **Angela K Chua**[1], **Obed Garcia**[5], **Yii-Der I Chen**[2,3], **Ronald M Krauss**[6], **Jerome I Rotter**[3,4], **Wendy Ankener**[7], **Richard S Legro**[8], **Ricardo Azziz**[2,4,*], **Jerome F Strauss III**[9], **Andrea Dunaif**[5], and **Margrit Urbanek**[5]

[1]Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA

[2]Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, Los Angeles, CA, USA

[3]Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

[4]Department of Medicine, the David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

[5]Division of Endocrinology, Metabolism, and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

[6]Children's Hospital Oakland Research Institute, Oakland, CA, USA

[7]Department of Human Genetics, University of Pennsylvania, Philadelphia, PA, USA

[8]Department of Obstetrics and Gynecology, Pennsylvania State College of Medicine, Hershey, PA, USA

[9]Department of Obstetrics and Gynecology, Virginia Commonwealth University, Richmond, VA, USA

Abstract

Background—Polycystic ovary syndrome (PCOS) is a complex endocrine disorder with a strong familial component. PCOS is characterized by hyperandrogenemia and irregular menses. A recent genome wide association study of PCOS in a Chinese cohort identified three reproducible PCOS susceptibility loci mapping to 2p16.3 (luteinizing hormone/choriogonadotropin receptor; *LHCGR*), 2p21 (thyroid associated protein; *THADA*), and 9q33.3 (DENN/MADD domain containing 1A; *DENNDIA).* The impact of these loci in non-Chinese PCOS cohorts remains to be determined.

Methods/Results—We tested association with PCOS of seven single nucleotide polymorphisms mapping to the three Chinese PCOS loci in two European-derived PCOS cohorts (Cohort A = 939 cases and 957 controls; Cohort B = 535 cases and 845 controls). Cases fulfilled the NICHD criteria for PCOS. Variation in *DENND1A* was strongly associated with PCOS in our cohort

Corresponding author: Margrit Urbanek, Division of Endocrinology, Metabolism, and Molecular Medicine, Northwestern University School of Medicine, 303 E. Chicago Ave., Tarry 15-717, Chicago, IL 60611, Phone: 312-503-3658, Fax: 312-908-9032, m-urbanek@northwestern.edu.
*Currently, Office of the President, Georgia Health Sciences University, Augusta, GA

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COMPETING INTERESTS
None

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($p_{combined\ cohorts}=10^{-8}$); multiple variants in *THADA* were also associated with PCOS, while there was no significant evidence for association of *LHCGR* variation with PCOS. We had greater than 80% power to detect an effect of similar size as was observed by Chen et al. for *DENND1A* and *THADA* but reduced power (at <40%) for *LHCGR* at p=0.0001. We had sufficient power (57-88%) for *LHCGR* at p=0.01.

Conclusions—At least two of the PCOS susceptibility loci identified in the Chinese PCOS GWAS (*DENND1A* and *THADA*) are also associated with PCOS in European-derived populations, and therefore likely to be important in the etiology of PCOS regardless of ethnicity. Our analysis of the *LHCGR* gene was not sufficiently powered to detect modest effects.

Keywords

PCOS; *DENND1A*; *THADA*; genome-wide association study

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder of reproductive age women that is characterized by elevated androgens and oligomenorrhea and amenorrhea and is associated with an increased risk of developing insulin resistance, obesity, and type 2 diabetes. PCOS is a complex disorder with both environmental and genetic factors contributing to its etiology [1]. Although numerous attempts have been made to identify genetic susceptibility loci for PCOS, only a few have been reproducibly identified [2].

Recently, the first genome-wide association study (GWAS) of PCOS was published. This study identified three loci that were reproducibly associated with PCOS in a Chinese cohort [3]. These PCOS susceptibility loci mapped to the genomic areas of three genes, *LHCGR, THADA*, and *DENND1A. LHCGR* encodes luteinizing hormone/choriogonadotropin receptor, which is the receptor for two glycoprotein hormones, luteinizing hormone (LH) and human chorionic gonadotropin (hCG). LH stimulates ovarian theca cells to produce testosterone, which is then converted to estrogen by granulosa cells. The mid-cycle LH surge triggers ovulation, after which LH also stimulates the corpora lutea of the follicles to produce progesterone; hCG is required for the maintenance of pregnancy. FSHR is the receptor for follicle-stimulating hormone (FSH), which stimulates the development of ovarian follicles; the *FSHR* gene is adjacent to *LHCGR. THADA* encodes thyroid adenoma-associated protein, which is expressed in pancreas, adrenal medulla, thyroid, adrenal cortex, testis, thymus, small intestine, and stomach; chromosomal aberrations of the genomic region containing *THADA* have been observed in benign thyroid adenomas. SNPs within *THADA* have also been associated with type 2 diabetes [4]. *DENND1A* encodes a protein named DENN/MADD domain containing 1A or DENND1A, a member of the connecdenn family, which plays a role in Rab35-activated endocytotic trafficking.

While these three loci show reproducible evidence for association in the Chinese population, it is not known whether they also contribute to PCOS susceptibility in non-Chinese populations. In this study we sought to replicate the three PCOS susceptibility loci identified by GWAS in the Chinese population in PCOS case/control cohorts of European ancestry. We tested for association between PCOS and one single nucleotide polymorphism (SNP) in *LHCGR*, four SNPs in *THADA*, and two SNPs in *DENND1A* in two cohorts consisting in total of 1474 women with PCOS and 1802 female controls. These seven SNPs were among the most significant associations in the Chinese GWAS, and *THADA* and *DENND1A* variants are also associated with PCOS in women of European ancestry.

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PATIENTS AND METHODS

Subjects

This study was approved by the Institutional Review Boards of the Brigham and Women's Hospital, Northwestern University Feinberg School of Medicine, Pennsylvania State University (PSU) College of Medicine, and University of Pennsylvania Medical Center, University of Alabama at Birmingham (UAB), Cedars-Sinai Medical Center (CSMC), Virginia Commonwealth University, the Pregnancy in PCOS (PPCOS) trial [5], and the Cholesterol and Atherosclerosis Pharmacogenetics (CAP) study [6]. Written informed consent was obtained from all participants.

Cohort A—The first case control cohort (no overlap with Cohort B) consisted of 939 index cases (probands) with PCOS and 957 control women (109 intensively phenotyped subjects recruited in parallel to the PCOS probands and 848 minimally phenotyped from a DNA repository) of European Caucasian ancestry. Subjects were recruited at Brigham and Women's Hospital, Northwestern University, and Pennsylvania State University. Phenotypic characteristics of cases and controls are given in Table 1.

PCOS cases: PCOS was defined according to the classic NICHD criteria and as previously implemented by us [7, 8, 9, 10]. All women with PCOS had hyperandrogenemia and chronic anovulation with the exclusion of specific disorders of the ovaries, adrenal, or pituitary [7, 8, 9, 10] and therefore satisfy the NICHD, Rotterdam, and Androgen Excess Society criteria for the diagnosis of PCOS [11, 12, 13].

Controls: Intensively phenotyped reproductively normal control women (n=109) were phenotyped as previously reported [8, 10]. They had normal androgen levels, regular menses, and were of similar age, weight, and ethnicity to the PCOS cases. To increase the number of control subjects, we used minimally phenotyped women (n=848) selected from NUgene, a large scale genebank (http://www.nugene.org) that combines a centralized genomic DNA sample collection and storage system with the ability to update participants' health status from electronic medical records. The majority of the minimally phenotyped subjects (>80%) were queried by questionnaire, had no history of irregular menses, and did not carry a diagnosis of PCOS. We, therefore, expect that our control cohort will include fewer women with PCOS than the population prevalence of PCOS (5-10%) [14].

Study protocols: None of the intensively phenotyped subjects were receiving medications known to alter reproductive hormone levels or glucose homeostasis for at least one month prior to study. Contraceptive steroids were stopped at least three months prior to study. Anthropometric measurements (weight and height) were taken as reported [15]. Circulating levels of total testosterone (T), non-sex hormone-binding globulin-bound testosterone (uT), and dehydroepiandrosterone sulfate (DHEAS) were determined from a fasting blood sample [7, 8].

Cohort B—The second case control cohort (no overlap with Cohort A) consisted of 535 unrelated white PCOS patients and 845 white control women. Sources of subjects included UAB (241 PCOS and 147 controls), CSMC (179 PCOS and 27 controls), PSU (recruited by R. S. Legro separately from above; 46 PCOS and 68 controls) [7], the PPCOS trial (69 cases) [5], and the CAP study (603 controls) [6]. All PCOS subjects met 1990 NIH criteria [13]. Parameters for defining hirsutism, hyperandrogenemia, ovulatory dysfunction, and exclusion of related disorders were previously reported for the UAB and CSMC [14], the additional PSU [7], and the PPCOS [5] samples. The CAP samples (271 women and 332

men) consist of general community controls. Table 1 presents clinical characteristics of Cohort B.

Genotyping

Genotyping in Cohort A was carried out using the Applied Biosystems Assays by Design (ABD) or Assays on Demand (AOD) 5′nuclease Taqman technology (Applied Biosystems, Foster City, CA) as recommended by the manufacturer on the 7900HT DNA analysis system (Applied Biosystems). Duplicate genotyping of a HapMap CEU trio yielded a 99.7% concordance rate. The genotyping success rate was 97%. All SNPs were in Hardy-Weinberg equilibrium. We genotyped seven markers, consisting of the SNPs most associated with PCOS in the Chinese GWAS or proxies for these SNPs (Table 2). Rs13405728 maps to 2p13.6 (*LHCGR*), rs12468394, rs13429458, rs6544661, and rs11891936 map to 2p21 (*THADA)*, and rs2479106 and rs10818854 map to 19q33.3 (*DENND1A).* Rs6544661 was used as a proxy for rs12478601 with which it is in complete LD (r^2=1) in the CEU population of the HapMap database (release 24, http://hapmap.ncbi.nlm.nih.gov/). A third SNP, rs10986105, associated with PCOS in the Chinese GWAS was not genotyped herein, because, unlike in Asian populations, in European populations it is in high LD with rs10818854 (r^2=0.83 in HapMap CEU). In some cases, technical reasons necessitated genotyping of proxy SNPs in linkage disequilibrium with the SNPs of interest; r^2 values in Table 2 are linkage disequilibrium (HapMap CEU) of the genotyped SNP with the Chinese GWAS SNP. Seven SNPs genotyped in each cohort captured the variation of the eight SNPs of interest.

In Cohort B, genotyping was carried out using iSelect Infinium technology, following the manufacturer's protocol (Illumina, San Diego, CA) [16, 17]. SNPs were excluded if the genotyping failure rate was >10% or if the minor allele frequency was <3%. Duplicate genotyping of 12 samples yielded a 100% concordance rate. The genotyping success rate was 99.97%. A total of seven SNPs were examined (Table 2), all of which were in Hardy-Weinberg equilibrium. In the 2p13.6 locus (*LHCGR*), we genotyped rs6732721, a proxy for rs13405728 (r^2 = 0.87 in HapMap CEU). In the 2p21 locus (*THADA*), we genotyped rs12468394, rs13429458, rs11891936, and rs6544661. In the 9q33.3 (*DENND1A*) locus, we genotyped rs2479106 and rs12337273, a proxy for two of the Chinese GWAS SNPs (r^2=0.83 with rs10818854 and r^2=1 with rs10986105 in HapMap CEU).

Statistical analysis

Unpaired t-tests were used to compare clinical characteristics between cases and controls; quantitative traits were log- or square-root-transformed as appropriate to reduce non-normality. Quantitative trait data are presented as median (interquartile range).

Separately in Cohorts A and B, association analyses were conducted using logistic regression; the dependent variable was PCOS status, and the independent variable was genotype (additive model). To assess whether the effects of the seven SNPs were independent of BMI, adjusted analyses were conducted with inclusion of BMI as an additional independent variable. Furthermore, we directly evaluated the seven SNPs for an effect on BMI by conducting linear regression wherein BMI was the dependent variable. Meta-analyses were conducted on the logistic regression results of the Cohort A and Cohort B using inverse variance weighting.

Power analysis—We used the Genetic Power Calculator package to calculate the power to detect an association between SNPs tested and PCOS in our cohort [18]. The allele frequencies in the power analysis are those for the PCOS associated allele (or corresponding proxy SNP alleles) at each SNP in the Hapmap CEU cohort (rs13405728 Allele G = 0.058;

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rs6732721 Allele C = 0.067; rs12468394 Allele A = 0.508; rs13429458 Allele C = 0.096; rs12478601 Allele T = 0.600; rs11891936 Allele A =0.200; rs6544661 Allele G = 0.600; rs2479106 Allele G = 0.300; rs10818854 Allele A = 0.050; rs10986105 Allele C = 0.042; rs12337273 Allele G = 0.034). Other parameters used for this analyses were: 957 controls, 939 cases for cohort A, 845 controls, 535 cases for cohort B, 1802 controls, 1474 cases for the complete cohort, genotype relative risk of 1.5 and 2.0 under an additive model. Assuming these parameters, we had 88% power to detect an effect at $p< 1\times10^{-4}$ in the complete cohort for at least one SNP mapping to *DENND1A* and *THADA* with genotype relative risks of 1.5 and 2.0. For *LHCGR* we had 57% power to detect an effect at p = 0.01 in the complete cohort for a genotype relative risk of 1.5 and >80% power detect an effect at p = 0.01 in the complete sample for a genotype relative risk of 2.0. We therefore had modest to sufficient power to detect a relevant effect in our cohort.

Quantitative trait association analyses—Exploratory association analyses of the seven SNPs against BMI, total testosterone, DHEAS, fasting insulin, and fasting glucose were conducted within each cohort. Because these analyses are not in replication of results from the Chinese GWAS, we applied a multiple testing corrected p value of 0.001 (=0.05/35; accounting for seven SNPs against five traits).

RESULTS

Allele frequencies and genotype frequencies for each SNP are shown in Table 3. In Cohort A, of the seven SNPs genotyped, four variants (three in *THADA*, one in *DENND1A*) were associated with PCOS (Table 4). These associations remained significant after adjustment for BMI (Table 5). The highest level of significance and greatest effect size were observed for the *DENND1A* SNP rs10818854. This was the only SNP associated with PCOS in Cohort B, with a similar odds ratio; adjustment for BMI did not materially alter this association (Tables 4 and 5). None of the seven SNPs was associated with BMI itself.

Meta-analysis results of the two cohorts are shown in Tables 4 and 5. The *DENND1A* SNP rs10818854 was highly associated with PCOS (unadjusted $P=9.8\times10^{-8}$, BMI-adjusted $P=6.5\times10^{-8}$). Three SNPs in the *THADA* locus, rs12468394, rs6544661, and rs11891936, were significantly associated with PCOS at lower levels of significance (Tables 4 and 5). The magnitude and direction of effects of the European PCOS associated *DENND1A* and *THADA* SNPs were similar to those observed in the Chinese GWAS (Table 2).

None of the seven SNPs were associated with total testosterone, DHEAS, fasting insulin, or fasting glucose (data not shown).

DISCUSSION

In this study, we performed a meta-analysis of two case control cohorts examining whether variants recently identified in a GWAS for PCOS in Chinese Han subjects would be associated with PCOS in non-Hispanic whites. Of the seven SNPs tested, one, rs10818854 in the *DENND1A* gene, was highly associated with PCOS in the present analysis; three SNPs in *THADA* were associated with PCOS in the meta-analysis. Finding variants in two of three genes associated with PCOS in both Chinese and Europeans is not unexpected, because the remarkably similar prevalence of PCOS around the globe suggests it might be an ancient disorder, for which the existence of common susceptibility genes and alleles in different races would be predicted [19]. Indeed, among the four SNPs associated with PCOS in both Chinese and Europeans, the same alleles were associated with PCOS with similar odds ratios.

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Until recently, the field of PCOS genetics was dominated by candidate gene studies, which examined over 100 genes of which only a few have been replicated [2, 20, 21, 22, 23, 24]. Given the modest success of candidate gene studies in PCOS, GWAS for this condition have been highly anticipated. The first was conducted in a cohort of cases and controls from China, with two levels of replication [3]. That study identified SNPs in three loci, at chromosomes 2p13.1, 2p21, and 9q33.3, as replicated loci for PCOS. Genes found at these loci include *LHCGR, THADA*, and *DENND1A*, respectively. The three *DENND1A* SNPs associated with PCOS in the Chinese GWAS, rs10818854, rs2479106, rs10986105, are independent ($r^2 < 0.7$) of each other in Asian populations [3]. Since in white populations, rs10818854 and rs10986105 are highly correlated ($r^2 = 0.83$ in HapMap CEU), we examined only the former in this study and found it to be associated with PCOS.

DENND1A (also known as connecdenn) encodes a protein involved in endosomal membrane trafficking [25]. Its N-terminus contains a DENN (differentially expressed in neoplastic versus normal cells) motif, which is found in many proteins but whose function is uncertain [26]. At the plasma membrane, DENND1A interacts with clathrin and the clathrin adaptor protein AP-1, via residues in the C-terminal end [27, 28]. DENND1A also functions as a guanine nucleotide exchange factor (GEF) for the small GTPase Rab35, serving to link Rab35 with clathrin-mediated endocytosis [28]. DENND1A has been found in neuronal clathrin-coated vesicles, where it plays a role in synaptic vesicle endocytosis [27]. DENND1A is ubiquitously expressed, with highest levels in kidney and brain [29]. Genetic variation in *DENND1A* has been associated with personality traits (rs7852296, $P=9 \times 10^{-6}$ [30]) and weakly with cleft lip/palate (rs1928482, P=0.03, in only one of multiple cohorts studied [31]). Because a protein such as this would be expected to affect diverse processes, it is not surprising to find it associated with PCOS, a syndrome characterized by dysfunction in multiple organ systems (ovary, adrenal, hypothalamus, pituitary, insulin-responsive tissues). It has been speculated that *DENND1A* may affect the development of PCOS via altered activity of endoplasmic reticulum aminopeptidase 1 [3].

While it is possible that *DENND1A* contributes directly to the PCOS phenotype, alternatively the positive association signal may be related to variation in another gene in linkage disequilibrium with the positive SNP. One such candidate gene encodes a microRNA, mIR60, which is co-localized with *DENND1A*. mIR601 up-regulates expression of actin cytoskeleton, down-regulates the Fas-induced apoptosis pathway, and represses nuclear factor-kappaB transcription factor-dependent reporter expression [32]. The control of these signalling pathways might directly or indirectly contribute to the PCOS phenotype.

DENND1A has a paralog, DENND1C, located on chromosome 19p13, a region that we previously suggested harbors a risk allele for PCOS [21]; however, SNPs in the *DENND1C* gene were not associated with PCOS (data not shown).

Multiple SNPs in the *THADA* locus were associated with PCOS. Of interest, rs7578597, a missense variant in *THADA*, was associated with type 2 diabetes in a large GWAS meta-analysis [4]. However, the *THADA* SNPs associated with PCOS in our data are not in LD with the diabetes variant in the HapMap and 1000 Genomes Project databases [33]. This resembles the situation with the gene *TCF7L2*, wherein different variants affect diabetes susceptibility and PCOS susceptibility [34].

Of the three genes considered, only *LHCGR* did not show association with PCOS in our European-derived cohorts. The SNP genotyped is rare in Europeans (minor allele frequency 0.05 in HapMap CEU) but common in Han Chinese (frequency 0.23 in HapMap) resulting in reduced power to detect association of this SNP with PCOS in our cohorts, as our meta-

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analysis effect size (OR 0.8) was similar to that observed in the Chinese study (OR 0.7) [3]. *LHCGR* might contain susceptibility SNPs for PCOS in whites not tagged by SNP rs13405728 in Chinese. A similar finding was observed in the gene *TCF7L2*, wherein different variants were associated with type 2 diabetes in whites and Chinese [35]. Alternatively, the LD pattern in the Caucasian populations may be sufficiently different from that in the Chinese so that the genotyped SNP is not detecting the same causal variant in the two populations. Comprehensive fine mapping is needed to evaluate this possibility in PCOS.

In conclusion, the GWAS era has finally arrived in PCOS genetics. By discovering loci that would not otherwise be considered in traditional candidate gene approaches, GWAS will open new avenues in genetic and physiologic research in PCOS. *DENND1A* and *THADA* appear to affect PCOS risk in at least two different racial groups.

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REFERENCES

1. Sam S, Dunaif A. Polycystic ovary syndrome: Syndrome XX? Trends Endocrinol Metab. 2003; 14:365–70. [PubMed: 14516934]
2. Urbanek M. The genetics of the polycystic ovary syndrome. Nat Clin Pract Endocrinol Metab. 2007; 3:103–11. [PubMed: 17237837]
3. Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y, Li Z, You L, Zhao J, Liu J, Liang X, Zhao X, Zhao J, Sun Y, Zhang B, Jiang H, Zhao D, Bian Y, Gao X, Geng L, Li Y, Zhu D, Sun X, Xu JE, Hao C, Ren CE, Zhang Y, Chen S, Zhang W, Yang A, Yan J, Li Y, Ma J, Zhao Y. Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. Nat Genet. 2011; 43:55–9. [PubMed: 21151128]
4. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, de Bakker PI, Abecasis GR, Almgren P, Andersen G, Ardlie K, Bostrom KB, Bergman RN, Bonnycastle LL, Borch-Johnsen K, Burtt NP, Chen H, Chines PS, Daly MJ, Deodhar P, Ding CJ, Doney AS, Duren WL, Elliott KS, Erdos MR, Frayling TM, Freathy RM, Gianniny L, Grallert H, Grarup N, Groves CJ, Guiducci C, Hansen T, Herder C, Hitman GA, Hughes TE, Isomaa B, Jackson AU, Jorgensen T, Kong A, Kubalanza K, Kuruvilla FG, Kuusisto J, Langenberg C, Lango H, Lauritzen T, Li Y, Lindgren CM, Lyssenko V, Marvelle AF, Meisinger C, Midthjell K, Mohlke KL, Morken MA, Morris AD, Narisu N, Nilsson P, Owen KR, Palmer CN, Payne F, Perry JR, Pettersen E, Platou C, Prokopenko I, Qi L, Qin L, Rayner NW, Rees M, Roix JJ, Sandbaek A, Shields B, Sjogren M, Steinthorsdottir V, Stringham HM, Swift AJ, Thorleifsson G, Thorsteinsdottir U, Timpson NJ, Tuomi T, Tuomilehto J, Walker M, Watanabe RM, Weedon MN, Willer CJ, Illig T, Hveem K, Hu FB, Laakso M, Stefansson K, Pedersen O, Wareham NJ, Barroso I, Hattersley AT, Collins FS, Groop L, McCarthy MI, Boehnke M, Altshuler D. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet. 2008; 40:638–45. [PubMed: 18372903]
5. Legro RS, Myers ER, Barnhart HX, Carson SA, Diamond MP, Carr BR, Schlaff WD, Coutifaris C, McGovern PG, Cataldo NA, Steinkampf MP, Nestler JE, Gosman G, Guidice LC, Leppert PC. The Pregnancy in Polycystic Ovary Syndrome study: baseline characteristics of the randomized cohort including racial effects. Fertil Steril. 2006; 86:914–33. [PubMed: 16963034]

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6. Simon JA, Lin F, Hulley SB, Blanche PJ, Waters D, Shiboski S, Rotter JI, Nickerson DA, Yang H, Saad M, Krauss RM. Phenotypic predictors of response to simvastatin therapy among African-Americans and Caucasians: the Cholesterol and Pharmacogenetics (CAP) Study. Am J Cardiol. 2006; 97:843–50. [PubMed: 16516587]
7. Legro RS, Driscoll D, Strauss JF, Fox J, Dunaif A. Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. Proc Natl Acad Sci USA. 1998; 95:14956–60. [PubMed: 9843997]
8. Sam S, Legro RS, Bentley-Lewis R, Dunaif A. Dyslipidemia and metabolic syndrome in the sisters of women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2005; 90:4797–802. [PubMed: 15899949]
9. Sam S, Sung YA, Legro RS, Dunaif A. Evidence for pancreatic beta-cell dysfunction in brothers of women with polycystic ovary syndrome. Metabolism. 2008; 57:84–9. [PubMed: 18078863]
10. Sam S, Legro RS, Essah PA, Apridonidze T, Dunaif A. Evidence for metabolic and reproductive phenotypes in mothers of women with polycystic ovary syndrome. Proc Nat Acad Sci USA. 2006; 103:7030–5. [PubMed: 16632599]
11. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, Witchel SF. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. J Clin Endocrinol Metab. 2006; 91:4237–45. [PubMed: 16940456]
12. The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004; 19:41–7. [PubMed: 14688154]
13. Zawadzki, J.; Dunaif, A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif, A.; Givens, JR.; Haseltine, FP.; Merriam, GR., editors. Polycystic Ovary Syndrome. Blackwell Scientific; Boston, MA: 1992. p. 377-84.
14. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab. 2004; 89:2745–9. [PubMed: 15181052]
15. Legro RS, Bentley-Lewis R, Driscoll D, Wang SC, Dunaif A. Insulin resistance in the sisters of women with polycystic ovary syndrome: association with hyperandrogenemia rather than menstrual irregularity. J Clin Endocrinol Metab. 2002; 87:2128–33. [PubMed: 11994352]
16. Gunderson KL, Kuhn KM, Steemers FJ, Ng P, Murray SS, Shen R. Whole-genome genotyping of haplotype tag single nucleotide polymorphisms. Pharmacogenomics. 2006; 7:641–8. [PubMed: 16768648]
17. Gunderson KL, Steemers FJ, Ren H, Ng P, Zhou L, Tsan C, Chang W, Bullis D, Musmacker J, King C, Lebruska LL, Barker D, Oliphant A, Kuhn KM, Shen R. Whole-genome genotyping. Methods Enzymol. 2006; 410:359–76. [PubMed: 16938560]
18. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics. 2003; 19:149–50. [PubMed: 12499305]
19. Azziz R, Dumesic DA, Goodarzi MO. Polycystic ovary syndrome: an ancient disorder? Fertil Steril. 2011; 95:1544–8. [PubMed: 20979996]
20. Stewart DR, Dombroski BA, Urbanek M, Ankener W, Ewens KG, Wood JR, Legro RS, Strauss JF, Dunaif A, Spielman RS. Fine mapping of genetic susceptibility to polycystic ovary syndrome on chromosome 19p13.2 and tests for regulatory activity. J Clin Endocrinol Metab. 2006; 91:4112–7. [PubMed: 16868051]
21. Urbanek M, Legro RS, Driscoll DA, Azziz R, Ehrmann DA, Norman RJ, Strauss JF, Spielman RS, Dunaif A. Thirty-seven candidate genes for polycystic ovary syndrome: strongest evidence for linkage is with follistatin. Proc Natl Acad Sci USA. 1999; 96:8573–8. [PubMed: 10411917]
22. Urbanek M, Woodroffe A, Ewens KG, Diamanti-Kandarakis E, Legro RS, Strauss JF, Dunaif A, Spielman RS. Candidate gene region for polycystic ovary syndrome on chromosome 19p13.2. J Clin Endocrinol Metab. 2005; 90:6623–9. [PubMed: 16091490]
23. Ewens KG, Stewart DR, Ankener W, Urbanek M, McAllister JM, Chen C, Baig KM, Parker SC, Margulies EH, Legro RS, Dunaif A, Strauss JF, Spielman RS. Family-based analysis of candidate

genes for polycystic ovary syndrome. J Clin Endocrinol Metab. 2010; 95:2306–15. [PubMed: 20200332]

24. Jones MR, Mathur R, Cui J, Guo X, Azziz R, Goodarzi MO. Independent confirmation of association between metabolic phenotypes of polycystic ovary syndrome and variation in the type 6 17beta-hydroxysteroid dehydrogenase gene. J Clin Endocrinol Metab. 2009; 94:5034–8. [PubMed: 19837928]
25. Yoshimura S, Gerondopoulos A, Linford A, Rigden DJ, Barr FA. Family-wide characterization of the DENN domain Rab GDP-GTP exchange factors. J Cell Biol. 2010; 191:367–81. [PubMed: 20937701]
26. Allaire PD, Marat AL, Dall'Armi C, Di Paolo G, McPherson PS, Ritter B. The Connecdenn DENN domain: a GEF for Rab35 mediating cargo-specific exit from early endosomes. Mol Cell. 2010; 37:370–82. [PubMed: 20159556]
27. Allaire PD, Ritter B, Thomas S, Burman JL, Denisov AY, Legendre-Guillemin V, Harper SQ, Davidson BL, Gehring K, McPherson PS. Connecdenn, a novel DENN domain-containing protein of neuronal clathrin-coated vesicles functioning in synaptic vesicle endocytosis. J Neurosci. 2006; 26:13202–12. [PubMed: 17182770]
28. Marat AL, Dokainish H, McPherson PS. DENN domain proteins: regulators of Rab GTPases. J Biol Chem. 2011; 286:13791–800. [PubMed: 21330364]
29. Nagase T, Kikuno R, Nakayama M, Hirosawa M, Ohara O. Prediction of the coding sequences of unidentified human genes. XVIII. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. DNA Res. 2000; 7:273–81. [PubMed: 10997877]
30. Verweij KJ, Zietsch BP, Medland SE, Gordon SD, Benyamin B, Nyholt DR, McEvoy BP, Sullivan PF, Heath AC, Madden PA, Henders AK, Montgomery GW, Martin NG, Wray NR. A genome-wide association study of Cloninger's temperament scales: implications for the evolutionary genetics of personality. Biol Psychol. 2010; 85:306–17. [PubMed: 20691247]
31. Letra A, Menezes R, Govil M, Fonseca RF, McHenry T, Granjeiro JM, Castilla EE, Orioli IM, Marazita ML, Vieira AR. Follow-up association studies of chromosome region 9q and nonsyndromic cleft lip/palate. Am J Med Genet A. 2010; 152A:1701–10. [PubMed: 20583170]
32. Ohdaira H, Nakagawa H, Yoshida K. Profiling of molecular pathways regulated by microRNA 601. Comput Biol Chem. 2009; 33:429–33. [PubMed: 19889580]
33. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics. 2008; 24:2938–9. [PubMed: 18974171]
34. Biyasheva A, Legro RS, Dunaif A, Urbanek M. Evidence for association between polycystic ovary syndrome (PCOS) and TCF7L2 and glucose intolerance in women with PCOS and TCF7L2. J Clin Endocrinol Metab. 2009; 94:2617–25. [PubMed: 19351735]
35. Goodarzi MO, Rotter JI. Testing the gene or testing a variant? The case of TCF7L2. Diabetes. 2007; 56:2417–9. [PubMed: 17901222]

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Table 1

Phenotypic characteristics of study subjects.

	Cohort A				Cohort B			
	PCOS (n=939)		Controls (n=957)		PCOS (n=535)		Controls (n=845)	
	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)
Age	939	28 (24-32)	957	36 (30-43)	535	27 (22-32)[a]	845	48 (36-58)
BMI (kg/m^2)	939	35.0 (29.1-41.5)[a]	957	24.0 (21.5-28.5)	535	31.7 (24.9-39.6)[a]	845	26.2 (23.3-30.4)
T(ng/dl)[b] [(mmol/liter)][c]	919	71 (59-90)[a] [2.5 (2.0-3.1)]	109	25 (19-36) [0.9 (0.7-1.2)]	521	67 (46-88)[a] [2.3 (1.6-3.1)]	219	34 (25-52) [1.2 (0.9-1.8)]
DHEAS (ng/ml)[d] [(|amol/liter)]	909	2076 (1446-2899)[a] [5.63 (3.92-7.87)]	109	1350 (935-1725) [3.66 (2.53-4.68)]	444[c]	2226 (1480-3271)[a] [6.04 (4.02-8.88)]	214	1070 (777-1565) [2.90 (2.11-4.25)]
Fasting insulin (μlU/ml) [(pmol/l)][c]	841	22 (15-33)[a] [132 (90-198)]	107	10 (8-13.5) [60 (48-81)]	428	14 (7-24)[a] [84 (42-144)]	676	11 (7.5-15.8) [66 (45-94.8)]
Fasting glucose (mg/dl) [(mmol/l)][c]	892	88 (83-95) [4.9 (4.6-5.3)]	108	89 (84-93) [4.9 (4.7-5.2)]	435	86 (81-92)[a] [4.8 (4.5-5.1)]	701	91.2 (85-98.8) [5.1 (4.7-5.5)]

IQR: interquartile range.

[a] P<0.0001 compared to controls within the same cohort

[b] Hormone assays for the two cohorts were carried out in different labs

[c] Conversion factors: T (ng/dl to mmol/liter) multiply by 0.03467; DHEAS (ng/ml to μmol/liter) multiply by 0.002714; insulin (μIU/ml to pmol/l) multiply by 6; glucose (mg/dl to mmol/l) multiply by 0.05551.

[d] DHEAS values were not available in the PPCOS subjects

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Table 2

Genotyping of SNPs most significantly associated with PCOS in the Chinese GWAS

Chr	Gene	Chinese GWAS SNP	Chinese associated allele	Chinese GWAS OR	SNP genotyped in Cohort A	SNP genotyped in Cohort B
2p13.6	*LHCGR*	rs13405728	G	0.71	rs13405728	rs6732721 (r^2=0.87)
2p21	*THADA*	rs12468394	A	0.72	rs12468394	rs12468394
2p21	*THADA*	rs13429458	C	0.67	rs13429458	rs13429458
2p21	*THADA*	rs12478601	T	0.72	rs6544661 (r^2=1)	rs6544661 (r^2=1)
2p21	*THADA*	rs11891936	A	0.66	rs11891936	rs11891936
9q33.3	*DENND1A*	rs2479106	G	1.34	rs2479106	rs2479106
9q33.3	*DENND1A*	rs10818854	A	1.51	rs10818854	rs12337273 (r^2=0.83)
9q33.3	*DENND1A*	rs10986105	C	1.47	rs10818854 (r^2=0.83)	rs12337273 (r^2=1)

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Table 3

Allele and genotype frequencies

			PCOS		Control	
SNP, allele	**Gene**	**Cohort**	**Allele freq**	**Genotype freq**	**Allele freq**	**Genotype freq**
rs12468394, A	*THADA*	A	0.469	0.221(AA) 0.497(AC) 0.282(CC)	0.519	0.268(AA) 0.502(AC) 0.230(CC)
		B	0.491	0.253(AA) 0.476(AC) 0.272(CC)	0.521	0.282(AA) 0.478(AC) 0.240(CC)
rs13429458, C	*THADA*	A	0.108	0.013(CC) 0.191(AC) 0.796(AA)	0.128	0.011(CC) 0.232(AC) 0.756(AA)
		B	0.121	0.015(CC) 0.211(AC) 0.774(AA)	0.111	0.015(CC) 0.192(AC) 0.793(AA)
rs6544661, G	*THADA*	A	0.569	0.195(AA) 0.471(AG) 0.333(GG)	0.605	0.146(AA) 0.498(AG) 0.356(GG)
		B	0.579	0.182(AA) 0.479(AG) 0.339(GG)	0.591	0.174(AA) 0.471(AG) 0.355(GG)
rs11891936, A	*THADA*	A	0.177	0.031(AA) 0.291(AG) 0.678(GG)	0.212	0.043(AA) 0.337(AG) 0.620(GG)
		B	0.184	0.041(AA) 0.286(AG) 0.673(GG)	0.198	0.050(AA) 0.296(AG) 0.654(GG)
rs2479106, G	*DENND1A*	A	0.316	0.100(GG) 0.431(AG) 0.469(AA)	0.310	0.094(GG) 0.432(AG) 0.474(AA)
		B	0.295	0.082(GG) 0.425(AG) 0.493(AA)	0.289	0.075(GG) 0.428(AG) 0.497(AA)
rs12337273, G	*DENN1DA*	B	0.060	0.006(GG) 0.109(GA) 0.886(AA)	0.030	0.000(GG) 0.059(GA) 0.941(AA)
rs10818854, A	*DENND1A*	A	0.073	0.004(AA) 0.137(AG) 0.859(GG)	0.046	0.003(AA) 0.086(AG) 0.911(GG)
rs6732721, C	*LHCGR*	B	0.059	0.004(CC) 0.110(CT) 0.886(TT)	0.066	0.004(CC) 0.124(CT) 0.872(TT)
rs13405728, G	*LHCGR*	A	0.043	0.002(GG) 0.083(AG) 0.915(AA)	0.056	0.004(GG) 0.103(AG) 0.892(AA)

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Table 4

Unadjusted genetic association analyses

		Cohort A			Cohort B			Meta-analysis		
SNP, allele	**Gene**	**OR**	**95% CI**	**P value**	**OR**	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
rs13405728, G	*LHCGR*	0.78	0.57-1.06	0.11	0.89	0.65-1.23	0.47	0.83	0.67-1.04	0.10
rs12468394, A	*THADA*	0.80	0.70-0.91	**0.0011**	0.89	0.77-1.04	0.13	0.84	0.76-0.93	**0.0006**
rs13429458, C	*THADA*	0.83	0.67-1.02	0.074	1.09	0.86-1.38	0.46	0.93	0.80-1.09	0.39
rs6544661, G	*THADA*	0.84	0.73-0.96	**0.013**	0.95	0.82-1.11	0.54	0.89	0.80-0.98	**0.022**
rs11891936, A	*THADA*	0.82	0.70-0.96	**0.024**	0.92	0.76-1.11	0.39	0.86	0.76-0.97	**0.017**
rs2479106, G	*DENND1A*	1.04	0.90-1.21	0.57	1.03	0.87-1.22	0.72	1.04	0.93-1.16	0.51
rs10818854, A	*DENND1A*	1.76	1.31-2.34	**0.00014**	2.08	1.43-3.04	**0.0002**	1.87	1.48-2.35	**9.8×10^{-8}**

Logistic regression models included PCOS status as the dependent variable, and genotype (additive model) as the independent variable. Rs6544661 is a perfect proxy for the Chinese GWAS PCOS-associated SNP rs12478601 (*THADA*). The meta-analysis was conducted on logistic regression models of Cohorts A and B. In Cohort B, rs6732721 was genotyped as a proxy for rs13405728 (*LHCGR*) and rs12337273 was genotyped as a proxy for rs10818854 (*DENND1A*); the correlated alleles were utilized in the meta-analysis. OR, odds ratio; 95% CI, 95% confidence interval.

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Table 5

BMI-adjusted genetic association analyses

		Cohort A			Cohort B			Meta-analysis		
SNP, allele	**Gene**	**OR**	**95% CI**	**P value**	**OR**	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
rs13405728, G	*LHCGR*	0.79	0.55-1.13	0.20	0.89	0.64-1.24	0.50	0.84	0.66-1.08	0.17
rs12468394, A	*THADA*	0.83	0.71-0.97	**0.017**	0.90	0.77-1.06	0.21	0.86	0.77-0.96	**0.0094**
rs13429458, C	*THADA*	0.90	0.70-1.15	0.39	1.09	0.85-1.40	0.51	0.99	0.83-1.18	0.90
rs6544661, G	*THADA*	0.83	0.71-0.98	**0.026**	0.96	0.82-1.13	0.68	0.90	0.80-1.0	0.056
rs11891936, A	*THADA*	0.81	0.66-0.99	**0.037**	0.91	0.74-1.11	0.37	0.86	0.74-0.99	**0.033**
rs2479106, G	*DENND1A*	1.01	0.85-1.20	0.89	1.02	0.85-1.22	0.83	1.01	0.90-1.15	0.82
rs10818854, A	*DENND1A*	2.04	1.47-2.84	**0.000024**	1.98	1.33-2.95	**0.0008**	2.02	1.56-2.60	**6.5×10^{-8}**

Logistic regression models included PCOS status as the dependent variable, and genotype (additive model) and BMI as independent variables. Rs6544661 is a perfect proxy for the Chinese GWAS PCOS-associated SNP rs12478601 (*THADA*). The meta-analysis was conducted on logistic regression models of Cohorts A and B. In Cohort B, rs6732721 was genotyped as a proxy for rs13405728 (*LHCGR*) and rs12337273 was genotyped as a proxy for rs10818854 (*DENND1A*); the correlated alleles were utilized in the meta-analysis. OR, odds ratio; 95% CI, 95% confidence interval

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