Published in final edited form as:

Fertil Steril. 2013 May ; 99(6): 1774–1778. doi:10.1016/j.fertnstert.2012.12.033.

Evaluating Reported Candidate Gene Associations with Polycystic Ovary Syndrome

Cindy Pau, M.D.¹, Richa Saxena, Ph.D.², and Corrine Kolka Welt, M.D.¹

¹Reproductive Endocrine Unit, Massachusetts General Hospital, Boston, MA

²Department of Anesthesia, Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA

Abstract

Objective—To replicate variants in candidate genes associated with PCOS in a population of European PCOS and control subjects.

Design—Case-control association analysis and meta-analysis.

Setting—Major academic hospital

Patients—Women of European ancestry with PCOS (n=525) and controls (n=472), aged 18 to 45 years.

Intervention—Variants previously associated with PCOS in candidate gene studies were genotyped (n=39). Metabolic, reproductive and anthropomorphic parameters were examined as a function of the candidate variants. All genetic association analyses were adjusted for age, BMI and ancestry and were reported after correction for multiple testing.

Main Outcome Measure—Association of candidate gene variants with PCOS.

Results—Three variants, rs3797179 (*SRD5A1*), rs12473543 (*POMC*), and rs1501299 (*ADIPOQ*), were nominally associated with PCOS. However, they did not remain significant after correction for multiple testing and none of the variants replicated in a sufficiently powered meta-analysis. Variants in the *FBN3* gene (rs17202517 and rs73503752) were associated with smaller waist circumferences and variant rs727428 in the *SHBG* gene was associated with lower SHBG levels.

Conclusion—Previously identified variants in candidate genes do not appear to be associated with PCOS risk.

Keywords

Candidate genes; genome-wide association

Corresponding author and person to who reprint requests should be addressed: Cindy Ta Pau, M.D. Reproductive Endocrine BHx 511 Massachusetts General Hospital 55 Fruit Street Boston, MA 02114 USA Fax: 617-726-5357 cta@partners.org.

Financial Disclosure: Cindy Pau, M.D. has nothing to disclose.

Richa Saxena, Ph.D. has nothing to disclose.

Corrine Kolka Welt, M.D. has nothing to disclose.

Clinical Trial Registration Number: NCT00166569

Introduction

Polycystic ovary syndrome (PCOS) affects 7-10% of reproductive age women, making it the most common endocrinopathy in this age group (1,2). Irregular menstrual cycles, hyperandrogenism and polycystic ovarian morphology are cardinal features of the syndrome (3). In addition, obesity and insulin resistance are common, along with an increased risk of diabetes, metabolic syndrome and other cardiovascular risk factors (4,5). Despite the detrimental impact on health, the etiology of PCOS is not understood.

A genetic approach has been taken to understand the etiology of PCOS. Twin studies suggest that the pathogenesis of PCOS is approximately 70% due to genetic influences (6). A genome-wide association study of Han Chinese women with PCOS demonstrated three PCOS risk loci, two of which were replicated in women of European descent (7-9). However, the majority of genetic studies in European women have used candidate gene linkage and association approaches that have not been replicated. Further, most of these studies suffered from small numbers and failure to correct for multiple testing, and therefore may represent false positive results (10). We aimed to replicate variants in candidate genes associated with PCOS in previous studies using genetic association analysis in European women with PCOS and control subjects and performed a meta-analysis using results from the existing literature.

Materials and Methods

Subjects

All subjects were U.S. women of European ethnicity and between the ages of 18 and 45 years. Subjects with PCOS ($n = 525$) had oligomenorrhea (< 9 menstrual periods/yr) and clinical and/or biochemical evidence of hyperandrogenism, fulfilling the NIH criteria (11,12). Clinical hyperandrogenism was defined by: 1) an elevated Ferriman Gallwey score > 9 or 2) acne on the face or back (11,13). Biochemical hyperandrogenism was defined as testosterone > 63 ng/mL (2.8 nmol/L), DHEAS > 430 μ g/dL (1.16 μ mol/L) or androstenedione levels > 3.8 ng/mL (13.3 nmol/L) (11). Control subjects ($n = 472$) had regular menstrual cycles, 21 to 35 days, and no reported or physical exam evidence of hyperandrogenism. All subjects were diagnosed before the age of 40 years and had been followed clinically in the authors' clinics prior to participation in the study. All subjects had normal TSH and prolactin levels, a follicular phase FSH level in the premenopausal range, and subjects with late onset congenital adrenal hyperplasia were excluded (11). Subjects were on no hormonal medication except for stable thyroid hormone replacement.

Protocol

The study was approved by the Institutional Review Board of the Massachusetts General Hospital and all subjects provided written informed consent. Subjects underwent a detailed history; physical exam including measurement of waist circumference at the umbilicus and hip circumference at the widest diameter; a pelvic ultrasound (ATL HDI 1500, 5 MHz convex array transducer); and blood samples for lipids, glucose, insulin, gonadotropin and sex-steroid levels, as described previously (11). An oral glucose tolerance test was performed with blood sampling 2 hours after a 75 gram glucose load. The anticipated differences between the PCOS cases and controls were present and reported previously (14).

Genotyping

Forty-three variants previously demonstrated to confer risk for PCOS in candidate gene association or linkage studies were selected for replication (Table 1). A panel of markers informative for European, African American and Latin ancestry was genotyped to control

for false associations related to population stratification (15-17). Patient DNA was isolated from whole blood. Genotyping was performed by primer extension of multiplex products with detection by MALDI-TOF mass spectroscopy using a Sequenom platform. Forty-four PCOS cases and 29 control samples failed with a call rate of <90%. Four SNPs failed with a call rate <90%. No additional SNPs deviated from Hardy-Weinberg equilibrium in cases or controls ($p > 10^{-3}$).

Statistical Analysis

PCOS case-control association analysis was performed using a logistic regression framework with SNPs coded using an additive genetic model. The primary association analysis included adjustment for age, BMI and four principal components (PCs) calculated by multidimensional scaling (MDS) analysis of identity-by-state distances of 152 unlinked ancestry-informative markers. Linear regression using an additive genetic model was used to test for association of potential PCOS risk variants with 30 log-transformed quantitative traits in PCOS cases, controls and the combined sample. Results adjusted for age, BMI and ancestry (15-17) are reported as P_{nominal} values and final P values are reported after correction for multiple testing by permutation analysis using 5000 permutations. A p value <0.05 was considered significant after corrections. Genetic association analyses were performed using PLINK (18). A meta-analysis was performed using previously published data combined with the current data using a Mantel-Haenszel model (19). Fourteen studies were included in the meta-analysis.

Results

Thirty-nine SNPs were successfully genotyped (Table 1). Three SNPs were found to be nominally associated with PCOS (rs3797179 located in an intron of the *SRD5A1* gene, rs12473543 located in exon 2 of the *POMC* gene and rs1501299 located in an intron of the *ADIPOQ* gene), but there was no evidence for association after correction for multiple testing.

Our meta-analysis of previously published data for PCOS variants showed adequate power to detect an association with PCOS in the majority of variants evaluated, including those in the *POMC* and *ADIPOQ* genes, but none were associated with PCOS (Table 1).

Although not found to be associated with PCOS, three variants were associated with quantitative traits after controlling for age, BMI, ancestry, and multiple testing between PCOS and control subjects. Two variants in the *FBN3* gene (rs17202517 and rs73503752) were associated with smaller waist circumferences and variant rs727428 in the sex hormone-binding globulin (SHBG) gene was associated with lower SHBG levels (Table 2).

Discussion

Only a few PCOS risk variants identified in genome-wide and candidate gene association studies have been examined in replication studies, with two loci emerging as PCO risk variants in European women (7-9). However, the importance of replicating additional candidate gene association results is paramount. In the current study, we tested variants previously demonstrated to confer risk for PCOS in candidate gene association and linkage studies. Although our sample size (525 cases and 472 controls) was larger than most of the included studies, we were not able to replicate the previously published results in the current study or in a meta-analysis sufficiently powered to examine the strongest loci.

The strongest previous replication study included 502 PCOS probands and used TDT methodology to demonstrate that variants in *FBN3* and *POMC* genes were over-transmitted

in PCOS (20). However, the allele that was over-transmitted had the opposite relationship to PCOS in the current study for both genes, and a meta-analysis that was sufficiently powered to detect an association demonstrated no significance. Variants in the *SRD5A1* and *ADIPOQ* genes were also nominally significant in the current study, but their associations failed to replicate with correction for multiple testing and did not prove to be significant in the sufficiently powered meta-analysis for these SNPs.

Our data replicate previous findings demonstrating that a polymorphism in the *SHBG* gene is associated with plasma levels of SHBG (21,22). Variant rs727428 was associated with lower mean SHBG levels in women with PCOS (21). Although not found to be associated with PCOS in our replication study or meta-analysis, two *FBN3* variants, rs17202517 and rs73503752, were found to be associated with a smaller waist circumference in both PCOS and control subjects. The *FBN3* gene encodes fibrillin-3, a structural protein of connective tissue (23), and a microsatellite in an intron of the gene has associated with unfavorable metabolic features, namely insulin resistance, in women with PCOS (24). It is possible that these two *FBN3* variants, which appear in lower allelic frequencies in cases compared to controls, are located in suppressor regions of the gene resulting in decreased connective tissue expression and lower waist circumference in carriers.

The study was limited by the possibility that real associations between the genes tested and PCOS were missed because we aimed to test a broad range of associations, and thus, not all variants in each gene were tested. Power was also a limitation as the number of cases and controls we identified could only demonstrate association in genes with an expected OR 1.5 and a risk allele frequency between 30-40%. However, our meta-analysis provided sufficient power to detect true evidence for association for many of the variants evaluated. This included variants that were significant in previous studies (20), as well as the 3 variants that were nominally significant before multiple testing in the current study. However, it is recognized that the power calculated using the first publication of an association may not be adequate due to the “winner’s curse,” which may result in an overestimation of the effect-size of the association (25).

In one of the largest replication cohorts and meta-analyses to date, the current study fails to replicate previous variants in candidate genes putatively associated with PCOS. Further collaboration and larger replication studies examining a wider range of variants will be essential to demonstrate genetic risk variants for PCOS.

Acknowledgments

Financial Support: This work was supported by the National Institutes of Health 1R01HD065029 (CKW), ADA 1-10-CT-57 (CKW), 1 UL1 RR025758 Harvard Clinical and Translational Science Center, M01-RR-01066 from the National Center for Research Resources.

Reference List

1. Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S, Escobar-Morreale HF. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. *J Clin Endocrinol Metab.* 2000; 85:2434–2438. [PubMed: 10902790]
2. 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; 89:2745–2749. [PubMed: 15181052]
3. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod.* 2004; 19:41–47. [PubMed: 14688154]

4. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care*. 1999; 22:141–146. [PubMed: 10333916]
5. Legro RS, Kusanman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J Clin Endocrinol Metab*. 1999; 84:165–169. [PubMed: 9920077]
6. Vink JM, Sadrzadeh S, Lambalk CB, Boomsma DI. Heritability of polycystic ovary syndrome (PCOS) in a Dutch twin-family study. *J Clin Endocrinol Metab*. 2005; 91:2100–2104. [PubMed: 16219714]
7. Welt CK, Stykarsdottir U, Ehrmann DA, Thorleifsson G, Arason G, Gudmundsson JA, et al. Variants in *DENND1A* are Associated with Polycystic Ovary Syndrome in Women of European Ancestry. In Revision. 2012
8. Goodarzi MO, Jones MR, Li X, Chua AK, Garcia OA, Chen YD, et al. Replication of association of *DENND1A* and *THADA* variants with polycystic ovary syndrome in European cohorts. *J Med Genet*. 2012; 49:90–95. [PubMed: 22180642]
9. Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y, et al. Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. *Nat Genet*. 2011; 43:55–59. [PubMed: 21151128]
10. Hattersley AT, McCarthy MI. What makes a good genetic association study? *Lancet*. 2005; 366:1315–1323. [PubMed: 16214603]
11. Welt CK, Arason G, Gudmundsson JA, Adams JM, Palsdottir H, Gudlaugsdottir G, et al. Defining constant versus variable phenotypic features of women with polycystic ovary syndrome using different ethnic groups and populations. *J Clin Endocrinol Metab*. 2006; 91:4361–4368. [PubMed: 16940441]
12. Zawadzki, JK.; 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: 1992. p. 377–384.
13. Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. *J Clin Endocrinol Metab*. 1961; 21:1440–1447. [PubMed: 13892577]
14. Saxena R, Welt CK. Polycystic ovary syndrome is not associated with genetic variants that mark risk of type 2 diabetes. *Acta Diabetol*. 2012
15. Price AL, Butler J, Patterson N, Capelli C, Pascali VL, Scarnicci F, et al. Discerning the ancestry of European Americans in genetic association studies. *PLoS Genet*. 2008; 4:e236. [PubMed: 18208327]
16. Price AL, Patterson N, Yu F, Cox DR, Waliszewska A, McDonald GJ, et al. A genomewide admixture map for Latino populations. *Am J Hum Genet*. 2007; 80:1024–1036. [PubMed: 17503322]
17. Smith MW, Patterson N, Lautenberger JA, Truelove AL, McDonald GJ, Waliszewska A, et al. A high-density admixture map for disease gene discovery in african americans. *Am J Hum Genet*. 2004; 74:1001–1013. [PubMed: 15088270]
18. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007; 81:559–575. [PubMed: 17701901]
19. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst*. 1959; 22:719–748. [PubMed: 13655060]
20. Ewens KG, Stewart DR, Ankener W, Urbanek M, McAllister JM, Chen C, et al. Family-based analysis of candidate genes for polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2010; 95:2306–2315. [PubMed: 20200332]
21. Wickham EP III, Ewens KG, Legro RS, Dunaif A, Nestler JE, Strauss JF III. Polymorphisms in the *SHBG* gene influence serum *SHBG* levels in women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2011; 96:E719–E727. [PubMed: 21252242]

22. Ding EL, Song Y, Manson JE, Hunter DJ, Lee CC, Rifai N, et al. Sex hormone-binding globulin and risk of type 2 diabetes in women and men. *N Engl J Med*. 2009; 361:1152–1163. [PubMed: 19657112]
23. Sabatier L, Miosge N, Hubmacher D, Lin G, Davis EC, Reinhardt DP. Fibrillin-3 expression in human development. *Matrix Biol*. 2011; 30:43–52. [PubMed: 20970500]
24. Urbanek M, Sam S, Legro RS, Dunaif A. Identification of a PCOS Susceptibility Variant in Fibrillin-3 and Association with a Metabolic Phenotype. *J Clin Endocrinol Metab*. 2007; 92:4191–4198. [PubMed: 17785364]
25. Xiao R, Boehnke M. Quantifying and correcting for the winner's curse in genetic association studies. *Genet Epidemiol*. 2009; 33:453–462. [PubMed: 19140131]
26. Graupp M, Wehr E, Schweighofer N, Pieber TR, Obermayer-Pietsch B. Association of genetic variants in the two isoforms of 5alpha-reductase, SRD5A1 and SRD5A2, in lean patients with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol*. 2011; 157:175–179. [PubMed: 21530059]
27. Xita N, Georgiou I, Chatzikyriakidou A, Vounatsou M, Papassotiriou GP, Papassotiriou I, et al. Effect of adiponectin gene polymorphisms on circulating adiponectin and insulin resistance indexes in women with polycystic ovary syndrome. *Clin Chem*. 2005; 51:416–423. [PubMed: 15590747]
28. Ranjzad F, Mahmoudi T, Irani SA, Mahban A, Nikzamir A, Vahedi M, et al. common variant in the adiponectin gene and polycystic ovary syndrome risk. *Mol Biol Rep*. 2012; 39:2313–2319. [PubMed: 21637951]
29. Escobar-Morreale HF, Villuendas G, Botella-Carretero JI, varez-Blasco F, Sanchon R, Luque-Ramirez M, et al. Adiponectin and resistin in PCOS: a clinical, biochemical and molecular genetic study. *Hum Reprod*. 2006; 21:2257–2265. [PubMed: 16675483]
30. San Millan JL, Corton M, Villuendas G, Sancho J, Peral B, Escobar-Morreale HF. Association of the polycystic ovary syndrome with genomic variants related to insulin resistance, type 2 diabetes mellitus, and obesity. *J Clin Endocrinol Metab*. 2004; 89:2640–2646. [PubMed: 15181035]
31. Goodarzi MO, Jones MR, Chen YD, Azziz R. First evidence of genetic association between AKT2 and polycystic ovary syndrome. *Diabetes Care*. 2008; 31:2284–2287. [PubMed: 18768676]
32. Goodarzi MO, Shah NA, Antoine HJ, Pall M, Guo X, Azziz R. Variants in the 5alpha-reductase type 1 and type 2 genes are associated with polycystic ovary syndrome and the severity of hirsutism in affected women. *J Clin Endocrinol Metab*. 2006; 91:4085–4091. [PubMed: 16849416]
33. Goodarzi MO, Antoine HJ, Pall M, Cui J, Guo X, Azziz R. Preliminary evidence of glycogen synthase kinase 3 beta as a genetic determinant of polycystic ovary syndrome. *Fertil Steril*. 2007; 87:1473–1476. [PubMed: 17270183]
34. Lee EJ, Oh B, Lee JY, Kimm K, Park JM, Baek KH. Association study between single nucleotide polymorphisms in the VEGF gene and polycystic ovary syndrome. *Fertil Steril*. 2008; 89:1751–1759. [PubMed: 17953946]
35. Kevenaar ME, Themmen AP, van Kerkwijk AJ, Valkenburg O, Uitterlinden AG, de Jong FH, et al. Variants in the ACVR1 gene are associated with AMH levels in women with polycystic ovary syndrome. *Hum Reprod*. 2009; 24:241–249. [PubMed: 18854405]
36. Mutharasan P, Galdones E, Bernabe BP, et al. Evidence for association of chromosome 2p16.3 polycystic ovary syndrome (PCOS) susceptibility locus in affected women of European ancestry. *Endocr Rev*. 2011; 32:P1–301.
37. Goodarzi MO, Maher JF, Cui J, Guo X, Taylor KD, Azziz R. FEM1A and FEM1B: novel candidate genes for polycystic ovary syndrome. *Hum Reprod*. 2008; 23:2842–2849. [PubMed: 18757445]
38. Goodarzi MO, Xu N, Cui J, Guo X, Chen YI, Azziz R. Small glutamine-rich tetratricopeptide repeat-containing protein alpha (SGTA), a candidate gene for polycystic ovary syndrome. *Hum Reprod*. 2008; 23:1214–1219. [PubMed: 18332089]
39. Urbanek M, Nampiaparampil G, D'Souza J, Sefton E, Ackerman C, Legro RS, et al. The role of genetic variation in the lamin a/c gene in the etiology of polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2009; 94:2665–2669. [PubMed: 19401371]

40. 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; 94:5034–5038. [PubMed: 19837928]
41. Jones MR, Wilson SG, Mullin BH, Mead R, Dudbridge F, Watts GF, et al. Polymorphism in postinsulin receptor signaling pathway is not associated with polycystic ovary syndrome. *Fertil Steril.* 2008; 90:2298–2303. [PubMed: 18249389]
42. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics.* 2003; 19:149–150. [PubMed: 12499305]

Table 1

Odds ratios (OR) for PCOS risk at loci identified in previous candidate gene association and linkage studies in the current study (Cases n=525; Controls n=472) and in a meta-analysis with published data.

Gene (ref)	Chr ^a	SNP ^b	Allele	Freq- Cases	Freq- Ctls	OR ^c (95% CI)	P _{nom} ^d	P _{adj} ^e	OR ^f (95% CI)	HWE ^g	PHWE ^h	P _{meta} ⁱ	# Cases Total ^j	# Cases Needed for 80% Power ^k
<i>SRD5A1</i> (20,26)	5	rs3797179	A	0.116	0.144	0.69 (0.49-0.98)	0.036	0.68	1.16 (0.88-1.53)	0.224	0.47	0.85	776*	167
<i>POMC</i> (20)	2	rs12473543	G	0.210	0.180	1.32 (1.01-1.73)	0.041	0.79	1.18 (0.98-1.41)	0.315	0.84	0.96	836*	374
<i>ADIPOQ</i> (20,27-30)	3	rs1501299	T	0.274	0.305	0.78 (0.62-0.99)	0.043	0.80	1.02 (0.89-1.17)	0.409	0.31	0.62	1269*	1047
<i>AKT2</i> (31)	19	rs3730051	C	0.251	0.296	0.80 (0.63-1.01)	0.064	0.91	0.96 (0.76-1.21)	0.397	0.57	0.36	814*	227
<i>SRD5A2</i> (32)	2	rs2754530	T	0.288	0.315	0.81 (0.64-1.03)	0.083	0.96	NA ^j	0.421	0.65	NA	NA	NA
<i>SRD5A2</i> (20)	2	rs765138	T	0.354	0.400	0.82 (0.65-1.03)	0.086	0.96	0.95 (0.82-1.11)	0.469	0.19	0.27	938	567/53
<i>GSK3B</i> (33)	3	rs1719895	A	0.080	0.073	0.68 (0.42-1.12)	0.13	0.99	NA ^j	0.142	1	NA	NA	NA
<i>FBN3</i> (20)	19	rs17202517	A	0.207	0.246	0.83 (0.64-1.08)	0.16	1	1.17 (0.98-1.39)	0.350	0.65	0.96	858*	318
<i>FBN3</i> (20)	19	rs73503752	T	0.209	0.246	0.84 (0.65-1.09)	0.19	1	1.18 (0.99-1.41)	0.351	0.24	0.97	848*	295
<i>VEGFA</i> (34)	6	rs6905288	G	0.379	0.419	0.88 (0.71-1.11)	0.28	1	NA ^j	0.480	0.39	NA	NA	NA
<i>ACVR1</i> (35)	2	rs10497189	C	0.115	0.106	1.20 (0.86-1.66)	0.28	1	1.01 (0.78-1.31)	0.198	0.11	0.53	886	18828

Gene (ref)	Chr ^a	SNP ^b	Allele	Freq- Cases	Freq- CIs	OR ^c (95% CI)	P _{nom} ^d	P _{adj} ^e	OR ^f (95% CI)	HWE ^g	PHWE ^h	P _{meta} ⁱ	# Cases Total ^j	# Cases Needed for 80% Power ^k
<i>ACVR2A</i> (20)	2	rs1424941	T	0.226	0.195	1.15 (0.88-1.50)	0.30	1	1.31 (1.08-1.59)	0.333	0.39	1	796*	423
<i>GSK3B</i> (33)	3	rs7624540	A	0.219	0.195	0.73 (0.39-1.38)	0.33	1	NA ^j	0.329	0.28	NA	NA	NA
<i>CYP11A</i> (20)	15	rs6495096	G	0.323	0.293	1.12 (0.89-1.41)	0.34	1	1.12 (0.93-1.34)	0.427	0.76	0.89	794	5380
<i>SHBG</i> (21)	17	rs6259	A	0.097	0.106	0.84 (0.59-1.21)	0.35	1	1.26 (0.97-1.64)	0.183	0.86	0.96	658	724
<i>LH</i> (36)	2	rs10495960	A	0.148	0.151	1.14 (0.84-1.54)	0.39	1	NA ^j	0.255	0.61	NA	NA	NA
<i>FEM1B</i> (20,37)	15	rs6494730	T	0.358	0.359	0.91 (0.73-1.13)	0.39	1	1.17 (1.01-1.35)	0.460	0.73	0.98	1204*	779
<i>GSK3B</i> (33)	3	rs1719889	A	0.228	0.196	0.78 (0.41-1.47)	0.44	1	NA ^j	0.334	0.25	NA	NA	NA
<i>SGTA</i> (20,38)	19	rs1005556	T	0.240	0.210	1.11 (0.85-1.44)	0.45	1	1.09 (0.91-1.30)	0.350	0.006	0.83	852*	803
<i>GSK3B</i> (33)	3	rs7620750	A	0.227	0.194	0.80 (0.42-1.51)	0.49	1	NA ^j	0.332	0.21	NA	NA	NA
<i>SGTA</i> (20,38)	19	rs2238614	A	0.232	0.207	1.10 (0.84-1.44)	0.49	1	1.05 (0.89-1.24)	0.343	0.26	0.72	1123*	442
<i>FEM1A</i> (20,37)	19	rs8111933	G	0.371	0.373	0.93 (0.75-1.16)	0.52	1	1.00 (0.88-1.14)	0.467	0.19	0.50	774	10343
<i>SGTA</i> (20,38)	19	rs1640262	C	0.247	0.222	1.09 (0.83-1.42)	0.54	1	1.08 (0.91-1.29)	0.360	0.07	0.81	1061*	250
<i>CYP11A</i> (20)	15	rs2277602	A	0.350	0.320	1.07 (0.86-1.34)	0.55	1	1.15 (0.96-1.37)	0.446	0.89	0.94	796	1671

Gene (ref)	Chr ^a	SNP ^b	Allele	Freq- Cases	Freq- CIs	OR ^c (95% CI)	P _{nom} ^d	P _{adj} ^e	OR ^f (95% CI)	HWE ^g	PHWE ^h	P _{meta} ⁱ	# Cases Total ^j	# Cases Needed for 80% Power ^k
<i>INSR</i> (20)	19	rs2252673	C	0.215	0.222	0.94 (0.73-1.22)	0.66	1	0.98 (0.84-1.14)	0.342	0.07	0.37	774	14128
<i>INSR</i> (20)	19	rs10401485	T	0.433	0.397	1.05 (0.85-1.30)	0.68	1	1.17 (0.98-1.39)	0.486	0.60	0.96	802	1360
<i>GSK3B</i> (33)	3	rs6770314	A	0.221	0.187	0.90 (0.49-1.65)	0.72	1	NA ^j	0.325	0.24	NA	NA	NA
<i>LMNA</i> (39)	1	rs2485664	T	0.096	0.087 ₂	0.94 (0.65-1.35)	0.73	1	NA ^j	0.166	0.12	NA	NA	NA
<i>ACVR2A</i> (20)	2	rs3768686	C	0.335	0.347	1.04 (0.83-1.30)	0.73	1	1.19 (1.02-1.40)	0.450	0.52	0.99	938*	638
<i>ACVR2A</i> (20)	2	rs3768688	A	0.378	0.399	1.04 (0.83-1.29)	0.74	1	1.12 (0.95-1.32)	0.476	0.54	0.91	862*	504
<i>FBN3</i> (20)	19	rs17160147	C	0.301	0.294	1.04 (0.83-1.30)	0.76	1	1.16 (0.99-1.36)	0.429	0.36	0.97	921*	861
<i>HSD17B6</i> (20,40,41)	12	rs898611	C	0.369	0.357	0.99 (0.80-1.23)	0.93	1	1.04 (0.91-1.19)	0.463	0.12	0.70	1461*	170
<i>GSK3B</i> (33)	3	rs2319398	A	0.416	0.373	0.99 (0.68-1.43)	0.95	1	NA ^j	0.478	0.35	NA	NA	NA
<i>GSK3B</i> (33)	3	rs6805251	T	0.418	0.368	1.007 (0.69-1.47)	0.97	1	NA ^j	0.478	0.38	NA	NA	NA
<i>ACVR1</i> (35)	2	rs1220134	A	0.295	0.299	1.00 (0.79-1.27)	0.98	1	1.03 (0.86-1.23)	0.419	0.70	0.62	886	12079
<i>GSK3B</i> (33)	3	rs2199503	T	0.271	0.306	1.00 (0.76-1.28)	0.98	1	NA ^j	0.411	0.35	NA	NA	NA
<i>SHBG</i> (20,21)	17	rs727428	T	0.427	0.424	1.00 (0.81-1.24)	0.98	1	1.02 (0.87-1.20)	0.489	0.65	0.61	889	9957

Gene (ref)	Chr ^a	SNP ^b	Allele	Freq- Cases	Freq- Cfs	OR ^c (95% CI)	P _{nom} ^d	P _{adj} ^e	OR ^f (95% CI)	HWE ^g	PHWE ^h	P _{meta} ⁱ	# Cases Total ^j	# Cases Needed for 80% Power ^k
<i>SHBG</i> (20,21)	17	rs6257	C	0.107	0.107	1.00 (0.70-1.42)	1.00	1	1.17 (0.90-1.53)	0.191	0.87	0.88	654	1000
<i>LMNA</i> (39)	1	lma24	0	NA	NA	NA	NA	1	NA	1	NA	NA	NA	NA

^aChr – chromosome

^bFour SNPs failed genotyping: rs1381841 (*GSK3B*), rs692243 (*PRKAG2*), rs8100018 (*AKT2*) and rs10152450 (*FEM1B*)

^cOR (95% CI) current study – odds ratio and 95% confidence interval

^dP_{nom} – nominal p value adjusted for age, BMI and ancestry

^eP_{adj} – p value corrected for multiple comparisons

^fOR (95% CI) meta-analysis – odds ratio and 95% confidence interval

^gHWE – Hardy Weinberg Equilibrium analysis

^hPHWE – Hardy Weinberg Equilibrium p value

ⁱP_{meta} –p value from meta-analysis

^jCases Total – total number of cases

^kCases Needed – total number of cases needed for 80% power to detect an association at the odds ratio initially defined in the literature (42);

* adequate power

^lData for individual variant not available in the literature or too rare

NA= not available or unable to calculate

Table 2

Quantitative traits associated with candidate variants in women with PCOS plus controls.

Gene	SNP	Group	Trait	Allele	Beta	P _{adj}
SHBG	rs727428	PCOS & Controls	SHBG level (nmol/L)	T	-0.0441	0.03
FBN3	rs17202517	PCOS & Controls	Waist Circumference (cm)	A	-0.0076	0.01
FBN3	rs73503752	PCOS & Controls	Waist Circumference (cm)	T	-0.0072	0.02

^aP_{adj} – p value controlled for age and BMI and corrected for multiple comparisons