Evaluating reported candidate gene associations with polycystic ovary syndrome

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Objective: To replicate variants in candidate genes associated with polycystic ovary syndrome (PCOS) in a population of European women with PCOS and control subjects.
Design: Case–control association analysis and meta-analysis.
Setting: Major academic hospital.
Patient(s): Women of European ancestry with PCOS (n = 525) and controls (n = 472), aged 18–45 years.
Intervention(s): 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, body mass index, and ancestry and were reported after correction for multiple testing.
Main Outcome Measure(s): Association of candidate gene variants with PCOS.
Result(s): 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 sex hormone-binding globulin levels.
Conclusion(s): Previously identified variants in candidate genes do not seem to be associated with PCOS risk.
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Key Words: Candidate genes, genome-wide association

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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.

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MATERIALS AND METHODS

Subjects

All subjects were US women of European ethnicity and between the ages of 18 and 45 years. Subjects with

PCOS (n = 525) had oligomenorrhea (fewer than nine menstrual periods per year) and clinical and/or biochemical evidence of hyperandrogenism, fulfilling the National Institutes of Health 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 T >63 ng/mL (2.8 nmol/L), DHEAS >430 μg/dL (1.16 μmoL/L), or androstenedione levels >3.8 ng/mL (13.3 nmol/L) (11). Control subjects (n = 472) had regular menstrual cycles, 21–35 days, and no reported or physical examination evidence of hyperandrogenism. All subjects were diagnosed before the age of 40 years and had been followed clinically in the authors' clinics before participation in the study. All subjects had normal TSH and PRL levels, a follicular phase FSH level in the premenopausal range, and subjects with late-onset congenital adrenal hyperplasia were excluded (11). Subjects were taking 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 examination 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-g 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 matrix-assisted laser desorption/ionization–time of flight mass spectroscopy using a Sequenom platform. Forty-four PCOS cases and 29 control samples failed, with a call rate of <90%. Four single nucleotide polymorphisms failed, with a call rate <90%. No additional SNPs deviated from Hardy-Weinberg equilibrium in cases or controls ($P>10^{-3}$).

Statistical Analysis

Polycystic ovary syndrome 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, body mass index (BMI), and four principal components calculated by multidimensional scaling 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 5,000 permutations. A P value <.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-Haenzel 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 present 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 present study or in a meta-analysis sufficiently powered to examine the strongest loci.

The strongest previous replication study included 502 PCOS probands and used the transmission disequilibrium test (TDT) methodology to demonstrate that variants in *FBN3* and *POMC* genes were overtransmitted in PCOS (20). However, the allele that was overtransmitted had the opposite