Association of Fibrillin-3 and Transcription Factor-7-Like 2 Gene Variants With Metabolic Phenotypes in PCOS

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Polycystic ovary syndrome (PCOS) is a complex genetic disease characterized by heritable reproductive and metabolic abnormalities. Genetic variants associated with the reproductive phenotype have been mapped to the fibrillin-3 (FBN3) gene and to a novel transcription factor-7-like 2 (TCF7L2) locus (rs11196236 G). The association of these genetic variants with metabolic phenotypes was investigated in 31 PCOS and 18 control women of European ancestry. The insulinogenic index during an oral glucose tolerance test (ΔI30/ΔG30) and insulin secretion rates at the maximal dose during a graded-glucose infusion (ISRmax) were used as indexes of insulin secretion. Endogenous glucose production (EGP) and insulin sensitivity (M/I) were determined during a euglycemic clamp. The disposition index (DI) was calculated using M/I and ΔI30/ΔG30 or ISRmax. Compared with noncarriers (n = 10) and control (n = 10), M/I was decreased (P = 1.1 × 10−5) in heterozygous and homozygous PCOS carriers (n = 14) of rs11196236 G and this variant predicted M/I (partial r2 = 0.34, P = 0.005) in a regression analysis. Postabsorptive EGP tended to be higher (P = 0.040) in heterozygous and homozygous PCOS carriers of the FBN3-associated allele (n = 12), allele 8 of D19S884 (FBN3+), compared to PCOS noncarriers (n = 19). PCOS carriers of the rs12255372 T (TCF7L2 Caucasian type 2 diabetes mellitus (T2D) locus) had no significant associated metabolic phenotypes. We conclude that rs11196236 G TCF7L2 variant is associated with peripheral insulin resistance in PCOS but this effect is not seen in control women. The FBN3 risk allele may be associated with changes in basal glucose homeostasis in PCOS. These findings require replication in additional PCOS cohorts.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting ~7% of premenopausal women (1,2). It is diagnosed by its reproductive phenotype of hyperandrogenism, chronic anovulation, and polycystic ovary morphology (3). However, it is now clear that PCOS is also an important metabolic disorder associated with insulin resistance and pancreatic β-cell dysfunction that confer a substantially increased risk for metabolic syndrome (4) and type 2 diabetes mellitus (T2D) (5) in affected women. PCOS is a highly heritable complex genetic disease (6). Male as well as female first-degree relatives have reproductive and metabolic phenotypes (7).

We previously mapped a genetic susceptibility variant associated with the PCOS reproductive phenotype to a dinucleotide repeat marker, D19S884, within intron 55 of the fibrillin-3 (FBN3) gene (8). Women with PCOS and one or two alleles of this variant (FBN3+) have significantly increased fasting insulin levels and homeostasis model assessment of insulin resistance values suggesting that they are more insulin resistant than affected women with all other alleles of D19S884 (FBN3−) (6). In contrast, FBN3+ brothers of women with PCOS have increased proinsulin:insulin molar ratios suggesting that they have pancreatic β-cell dysfunction (6). We have also found that a novel region of the diabetes susceptibility gene, transcription factor-7-like 2 (TCF7L2) (9), is associated with the PCOS reproductive phenotype (10). This region is not in linkage disequilibrium with the T2D susceptibility region that maps to introns 3 and 4 of TCF7L2 (9,10). However, the Caucasian T2D susceptibility region, delimited by the single-nucleotide polymorphisms (SNPs) rs4506565, rs7896811, rs11196192, rs11196199, rs1765538, rs7895340, and rs12255372, is associated with proinsulin:insulin molar ratios in women with PCOS.

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who have dysglycemia (10). This observation suggests that the TCF7L2 T2D locus is associated with markers of pancreatic β-cell dysfunction in PCOS as it is in other groups at risk for T2D (11).

Variants associated with complex genetic diseases are often in noncoding portions of the gene, as is the case with the variants in FBN3 and TCF7L2, and it has been difficult to determine their functional significance with traditional molecular biologic techniques (12). One approach to gain insight into the physiologic relevance of these variants is to examine genotype–phenotype associations (11). Since our study using fasting markers of glucose homeostasis suggested that the FBN3 variant is associated with insulin resistance and β-cell dysfunction (8), we performed a detailed assessment of insulin action and secretion to further investigate the impact of variation in FBN3 on these parameters. We also investigated the impact of the variants in TCF7L2 associated with PCOS and with T2D on insulin action and secretion in PCOS.

METHODS AND PROCEDURES
Thirty-one Caucasian women of European ancestry with PCOS and 18 reproducibly normal control women of comparable age, weight, and race/ethnicity were studied. All women were between the ages of 18–40 years, in good health, weight stable, and sedentary (<30 min per day of moderate physical activity). None of the women was taking any medications known to affect gonadal function or carbohydrate metabolism for at least 1 month before the study except for oral contraceptives, which were stopped at least 3 months before study. The studies were approved by the institutional review board of the Feinberg School of Medicine and written informed consent was obtained from all subjects before the study.

PCOS was diagnosed according to the National Institute of Child Health and Human Development criteria (3). All women with PCOS had hyperandrogenemia and chronic anovulation with the exclusion of specific disorders of the ovaries, adrenal, or pituitary glands (7). Control women were defined as having regular menstrual cycles every 27–35 days, Ferriman and Galloway scores of ≤8, and normal circulating androgen levels. Control women were selected to have comparable age and BMI to the women with PCOS. Because there are no significant differences in euglycemic clamp measures of insulin sensitivity (M/I) during the menstrual cycle (13,14), metabolic studies were performed without regard to the phase of the menstrual cycle in the morning after a 3-day 300-g carbohydrate preparatory diet and an overnight fast.

Body composition and visceral adiposity
Body composition was determined by dual photon X-ray absorptiometry (Hologic, Bedford, MA). Visceral adipose tissue was determined using a single computerized tomography slice at the L2–3 vertebral interspace (15).

Oral glucose tolerance test
A 75-g oral glucose tolerance test (OGTT) was performed with glucose and insulin levels obtained at 0 and 120 min in all subjects and every 30 min for 180 min in 24 PCOS and 18 control women. Glucose tolerance was classified based on the 120-min post-challenge glucose level according to World Health Organization criteria (5). By definition, all control women had normal glucose tolerance. Seven women with PCOS had impaired glucose tolerance but none had diabetes mellitus.

Sequential multiple insulin dose euglycemic clamp
A sequential multiple insulin dose euglycemic clamp was performed as previously reported (16,17). Postabsorptive endogenous glucose production (EGP) and its suppression by insulin were assessed using a primed (6.5 mg/kg), continuous infusion of [6,6-2H]-glucose (Isotec, Miamisburg, OH), at a dose of 0.065 mg/kg/min, starting at ~180 min (16). Starting at −5 min (18), an infusion of the somatostatin analog, octreotide (Sandostatin, Novartis Pharmaceuticals, East Hanover, NJ) at a dose of 30 ng/kg/min was begun to suppress endogenous insulin secretion so the insulin levels could be matched across the study groups (18). Sequential primed insulin doses of 5 (nonobese), 10 (obese), and 20 mU/m2/min were administered for 150 min per dose followed by a dose of 400 mU/m2/min for 90 min, which we have shown previously (16,17) maximally stimulates glucose uptake. The 5–20 mU/m2/min insulin doses were used to assess in vivo suppression of lipolysis and the results will be reported in a separate publication. Euglycemia was maintained with a variable infusion of 20% glucose enriched to ~2.5% with [6,6-2H]-glucose (16,19) to maintain constant isotopic enrichment. Arterialized blood was obtained every 5 min for glucose determinations, every 15 min for insulin levels, and every 10 min during the last 40 min of the baseline period and of each insulin dose for [6,6-2H]-glucose enrichment. Twenty-four PCOS and 10 control women received all clamp insulin doses, the clamps were ended early in the remaining subjects for technical reasons, mainly loss of intravenous access.

Graded-glucose infusion
Graded-glucose infusion was performed as previously reported (20) in a subset of 18 PCOS and 7 control women who had euglycemic clamp studies. Glucose was infused at 2, 4, 8, and 16 mg/kg/min for 40 min at each dose of glucose. Samples were collected every 10 min during the infusion for glucose, insulin, and C-peptide levels.

Genotyping
SNPs were genotyped using the Applied Biosystems Assays by Design 5'-nuclease TaqMan technology as recommended by the manufacturer and the 7900HT DNA analysis system (Applied Biosystems, Carlsbad, CA). The dinucleotide polymorphism D19S884 was genotyped as previously described (8). Based on our previous study (10), TCF7L2 rs11196236 G allele was selected for the PCOS locus. Two TCF7L2 SNPs that have been shown to have a strong effect in Caucasian populations with T2D (rs12255372 and rs7903146 in introns 3 and 4, respectively) are in linkage disequilibrium (9); we selected the rs12255372 T allele for the T2D locus as preliminary studies showed no differences in pheno- typic associations with this allele or the rs7903146 risk allele (data not shown). Both of the TCF7L2 SNPs had a minor allele frequency of at least 0.001. The number of homozygous and heterozygous women for each PCOS risk allele was: 1, 11 FBN3; 4, 15 rs11196236 G and 4, 14 rs12255372 T. The sample size of subjects was too small to examine the effects of gene dosage therefore, a dominant model was assumed and homozygous and heterozygous PCOS carriers of the risk allele were pooled in the genotype-positive group for the physiological studies. Homozygous and heterozygous PCOS carriers of the D19S884 FBN3 risk allele were designated as FBN3+ and women with all other alleles were denoted as FBN3−. Homozygous and heterozygous carriers of the TCF7L2 PCOS risk allele were designated by their allele, rs11196236 G and noncarriers were designated by their allele, rs11196236 A. Homozygous and heterozygous PCOS carriers of the TCF7L2 T2D risk allele were designated by their allele, rs12255372 T and noncarriers were designated by their allele, rs12255372 C.

Analytic techniques
Levels of testosterone (T), bioavailable testosterone (uT), sex hormone binding globulin (SHBG), and dehydroepiandrosterone sulfate (DHEAS) were determined as previously reported (7). Insulin and C-peptide levels were measured using radioimmunoassay kits (Millipore, Billerica, MA). Arterialized blood glucose levels during the euglycemic clamp studies were assayed using a YSI Glucose Analyzer (YSI, Yellow Springs, OH). Glucose levels from the OGTT and graded- glucose infusion were determined with a Beckman Synchron CX3 Delta Clinical Analyzer (Beckman Coulter, Fullerton, CA). Plasma [6,6-2H]-glucose specific activity was measured using gas chromatogra- phy mass spectroscopy assay (Metabolic Solutions, Nashua, NH).
Intravenous glucose-mediated insulin secretion rates (ISR) were determined using a steady-state tracer kinetics (19). Basal glucose clearance was calculated by dividing postabsorptive EGP by postabsorptive glucose levels. At an insulin dose of 400 mU/m²/min, EGP is completely suppressed (16) so the steady-state glucose infusion rate is equal to insulin-mediated glucose disposal. Euglycemic clamp M/I was determined as insulin-mediated glucose disposal at the 400 mU/m²/min insulin dose divided by the steady-state plasma insulin at this dose (21). This insulin dose was selected based on our previous studies showing that maximal responsiveness to insulin of glucose disposal at this dose (21). This insulin dose was selected based on our previous studies showing that maximal responsiveness to insulin of glucose disposal at this dose (21).

Intravenous glucose-mediated insulin secretion rates (ISR) were determined by deconvolution of C-peptide kinetics (20) at all glucose doses. The disposition index (DI), which assesses insulin secretion in relation to M/I (22), was calculated as the product of M/I and (i) ISR at 16 mg/kg/min glucose (ISRmax) as the index of insulin secretion in response to intravenous glucose and (ii) the insulinogenic index (change in insulin from 0 to 30 min divided by change in glucose from 0 to 30 min (ΔI30/ΔG30)) during the OGTT as an index of insulin secretion in response to an oral glucose challenge. DI calculated as the product of M/I and oral-mediated glucose secretion was multiplied by 1,000.

This study was designed a priori to assess the differences in eight study endpoints (postabsorptive EGP, basal glucose clearance, M/I, basal insulin secretion rate (ISRb), M/I, (DI-I30ΔG30, DI-ISRmax, DI-DI-ΔI30ΔG30)) by genotype in women with PCOS. Control women were included in order to provide a point of reference for any observed changes in PCOS but they were not stratified by genotype since we have no evidence for an effect of PCOS susceptibility variants in reproductive normal women (10). Accordingly, parametric and nonparametric one-way ANOVA, depending on the normality of the data, was applied to compare PCOS women stratified by genotype: genotypePCOS vs. genotypePCOS vs. control. This analysis was performed separately for each genotype variant, i.e., FBN3, rs11196236, and rs12255372. Tukey's honestly significant different (HSD) post-hoc test was applied to determine which groups differed significantly. Because of the significant impact of rs11196236 G on M/I in PCOS, heterozygous, and homozygous control carriers of rs11196236 G were compared to control carriers of rs11196236 A. For the comparison of baseline clinical and biochemical features in PCOS vs. control, t-test or Wilcoxon rank sum test was applied, depending on the normality of the data.

As an exploratory aim, regression analyses were performed to determine genetic and clinical predictors of insulin action and secretion. The first model used the gene variants alone as independent variables. The dependent variables were postabsorptive EGP, glucose clearance, M/I as parameters of insulin action and basal ISR (ISRb), DI-DI-ΔI30ΔG30 and DI-ΔI30ΔG30 as parameters of insulin secretion. Second, a stepwise regression was performed using five known physiologic predictors for changes in glucose homeostasis (BMI, visceral adipose tissue, fasting insulin, fasting glucose, and 2-h post-challenge glucose) as independent variables to select those predictive of each dependent variable. A third model used the gene variants with addition of the physiologic predictors.

Data analyses used SAS version 9.2 (SAS Institute, Cary, NC). Log transformation was performed when necessary to achieve homogeneity of variance. Results were considered significant at α = 0.05 in Table 1. The level of a was adjusted since eight endpoints were examined for three loci (8 × 3 = 24, P = 0.05/24 = 0.0021) for the analyses stratified by genotype; the ANOVA adjusted for the multiple comparisons of the genotypes at each locus. The nominal P (Pn) values are reported. Data in the text and tables are reported as mean ± s.d. and in the figure as mean ± s.e.m.

**RESULTS**

**Characteristics of the study population**

The characteristics of the subjects are summarized in (Table 1). The women with PCOS and control women were of comparable age and BMI by design. The groups also had comparable lean and fat mass by dual photon X-ray absorptiometry and visceral adiposity by computerized tomography. Levels of T, uT, and DHEAS were significantly increased in PCOS compared to control, consistent with the biochemical profile of PCOS (3). There were no significant differences in anthropometric parameters, body composition or androgen levels when the PCOS women were stratified by genotype (data not shown).

**Insulin action**

Postabsorptive EGP was nominally significantly higher in FBN3PCOS (n = 12) compared to FBN3PCOS (n = 18) but did not differ from that in control women (n = 18) women (81 ± 8 FBN3PCOS vs. 74 ± 12 FBN3PCOS vs. 78 ± 18 control mg/m²/min, ANOVA Pn = 0.040). Basal glucose clearance was also higher in FBN3PCOS compared to control but the difference did not achieve statistical significance (ANOVA Pn = 0.063). There was no difference in either postabsorptive EGP or basal glucose clearance when analyzed by rs12255372 or rs11196236 genotype. M/I was significantly decreased (ANOVA Pn = 1.1 × 10−5) in rs11196236 G (n = 14) compared to rs11196236 A (n = 10) (HSD Pn = 0.040) and to control women (n = 10) (HSD Pn = 3.3 × 10−4), but rs11196236 A did not differ from control women (Figure 1). M/I also did not differ when stratified by FBN3 or rs12255372 but was significantly decreased in both PCOS FBN3 and both rs12255372 genotype groups compared to control women (ANOVA Pn = 0.0008 and Pn = 0.0009, respectively), consistent with a PCOS effect to reduce M/I (Figure 1). M/I did not differ in control women stratified by rs11196236 genotype (data not shown).

**Table 1 Clinical and biochemical features**

| Variable | PCOS (n = 31) | Control (n = 18) | P value |
|----------|---------------|-----------------|---------|
| Age (years) | 29 ± 4a | 31 ± 5a | 0.30b |
| Weight (kg) | 92.0 ± 22.6a | 89.2 ± 18.5a | 0.66c |
| BSA (m²) | 2.03 ± 0.27a | 2.01 ± 0.21a | 0.76c |
| BMI (kg/m²) | 34.2 ± 7.8a | 33.0 ± 7.5a | 0.56a |
| VAT (cm²) | 98 ± 52a | 81 ± 43a | 0.29a |
| FFM (kg) | 54.0 ± 9.4a | 54.0 ± 7.6a | 0.99a |
| Fat (kg) | 37.9 ± 16.1a | 35.3 ± 11.7a | 0.79a |
| T (ng/dl) | 70 ± 27a | 28 ± 11a | <0.0001b |
| uT (ng/dl) | 25 ± 11a | 9 ± 4a | <0.0001d |
| DHEAS (mg/dl) | 3,237 ± 1,135a | 1,450 ± 531a | 0.001e |
| SHBG (nmol/l) | 45 ± 22a | 47 ± 29a | 0.85a |

*BSA, body surface area; DHEAS, dehydroepiandrosterone sulfate; FFM, fat-free mass; SHBG, sex hormone binding globulin; T, testosterone; uT, bioavailable testosterone; VAT, visceral adipose tissue.

| α = 0.05/24 = 0.0021) for the analyses stratified by genotype; cSample sizes 28 and 15, respectively. dWilcoxon rank sum test. eSample sizes 28 and 15, respectively.

**Insulin secretion**

ISRb was nominally significantly higher in FBN3PCOS compared to control women (326 ± 159 FBN3PCOS (n = 11) vs. 195 ± 69 FBN3PCOS (n = 7) vs. 173 ± 39 (n = 7) control pmol/min, ANOVA Pn = 0.019). ISRb did not differ when

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*HSD* post-hoc test. **Sample sizes 28 and 17, respectively. Wilcoxon rank sum test. Sample sizes 28 and 15, respectively.
be lower in rs12255372 T (n = 9) compared to PCOS women without the genotype (n = 7) (data not shown). DI calculated with either oral glucose-stimulated or intravenous glucose-stimulated insulin secretion did not differ in PCOS stratified by FBN3 or rs11196236.

**Regression analyses**

The regression analysis that included all genotypes showed that FBN3 risk allele was a nominally significant predictor of postabsorptive glucose clearance (partial $r^2 = 0.14$, $Pn = 0.049$) and that the rs12255372 risk allele was a nominally significant predictor of M/I (partial $r^2 = 0.34$, $Pn = 0.0046$). The only physiologic factors that were predictive of metabolic endpoints were BMI, visceral adipose tissue, fasting glucose, and post-challenge glucose. When these physiologic predictors were added to the model, the association of M/I with the rs11196236 risk allele remained nominally significant (partial $r^2 = 0.30$, $Pn = 0.010$).

**DISCUSSION**

We investigated whether variants in two genes, FBN3 and TCF7L2, that we have found to be associated with the PCOS reproductive phenotype were associated with metabolic phenotypes in affected women (8). Indeed, our prior studies using fasting parameters of glucose homeostasis suggested that women with PCOS of European ancestry who are homozygous and heterozygous carriers of the D19S884 PCOS susceptibility variant that maps to an intron of FBN3 are insulin resistant, whereas their male first-degree relatives who are homozygous and heterozygous carriers of this allele have evidence for pancreatic β-cell dysfunction (8). In the current study, comprehensive assessment of insulin action and secretion found no evidence for insulin resistance associated with the FBN3 PCOS risk allele. There was a trend towards alterations in postabsorptive glucose homeostasis in carriers of the FBN3 PCOS risk. In contrast, the TCF7L2 PCOS risk allele (rs11196236 G) was associated with substantial decreases in M/I. Exploratory regression analyses supported these findings by showing nominally significant associations of the TCF7L2 PCOS risk allele with M/I and the FBN3 PCOS risk allele with basal glucose clearance. The TCF7L2 T2D risk allele (rs12255372 T) was associated with a trend toward decreased insulin secretion. In the general population at risk for T2D (11) and in women with PCOS and dysglycemia (10), the TCF7L2 T2D risk allele is associated with markers of pancreatic β-cell dysfunction.

The decreases in M/I associated with the TCF7L2 PCOS risk allele were highly significant and remained significant after Bonferroni correction for multiple testing. Further, M/I did not differ significantly in the PCOS without the TCF7L2 PCOS risk allele compared to control women suggesting that the TCF7L2 PCOS risk allele was an important determinant of M/I in affected women. In addition, the TCF7L2 PCOS risk allele was a nominally significant predictor of M/I in regression analyses, independent of physiologic predictors of insulin action. In our previous study (10), the TCF7L2 PCOS risk allele was not associated with fasting parameters of insulin

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*Figure 1* M/I was significantly decreased in carriers of the TCF7L2 PCOS risk allele compared to noncarriers and to controls: rs11196236 G (n = 14) vs. rs11196236 A (n = 10), HSD $Pn = 0.0040$ and rs11196236 G (n = 14) vs. control (n = 10), HSD $Pn = 3.3 \times 10^{-4}$; top panel. M/I was significantly decreased in both PCOS groups stratified by the TCF7L2 T2D risk allele: rs12255372 T (n = 13) vs. control (n = 10), HSD $Pn = 1.7 \times 10^{-6}$ and in rs12255372 C (n = 11) vs. control (n = 10), HSD $Pn = 0.0198$, middle panel. M/I was significantly decreased in both PCOS groups stratified by the FBN3 risk allele: FBN3+ (n = 9) vs. control (n = 10), HSD $Pn = 0.0137$, FBN3− (n = 15) vs. control (n = 10), HSD $Pn = 2.4 \times 10^{-4}$, bottom panel. Data are mean ± s.e.m. HSD, Tukey’s honestly significant different; M/I, insulin sensitivity; PCOS, polycystic ovary syndrome; Pn, nominal P.
action. This observation supports the importance of intensive phenotyping for assessing the physiologic implications of genetic variation in complex diseases. In contrast, the TCF7L2 T2D risk allele was associated with a trend to decreased insulin secretion in PCOS.

These findings suggest that distinct metabolic phenotypes are found with variation in different regions of TCF7L2 in the same population of women with PCOS. A region in the TCF7L2 gene close to the PCOS susceptibility locus has recently been shown to be associated with fasting and OGTT parameters of insulin resistance in nondiabetic Taiwanese and Caucasian cohorts (23) supporting our observation that different regions of TCF7L2 have distinct effects on glucose homeostasis. However, there was no evidence for defects in insulin secretion associated with the Caucasian TCF7L2 T2D locus in that study, but this may be secondary to low minor allele frequencies seen in the Asian population (23).

TCF7L2 encodes for a transcription factor, which is one of the four T-cell transcription factors that serve as cofactors with free β-catenin to form a bipartite transcription factor that activates target genes in the wingless-type mouse mammary tumor virus integration site family (Wnt) signaling pathway (24). This pathway plays an important role in embryogenesis, gonadal development, self-renewal of adult tissues and cancer (25). TCF7L2 is widely expressed in human tissues including adipose tissue, gut, pancreas, liver, and brain (26, 27). TCF7L2 expression is decreased in islets (11) and adipose tissue (26) from T2D subjects. TCF7L2 effects on M/I could be mediated through its effects on adipose tissue (23). TCF7L2 is also expressed in the ovary and this expression is decreased in ovarian cumulus cells from lean women with PCOS (28). Several studies have found differential expression of members of the Wnt signaling pathway in PCOS ovaries (29) and omental adipose tissue (30). TCF7L2 protein has been shown to interact with the androgen receptor (31) and adolescent girls with Wnt 4 mutations can have hyperandrogenism (32). These observations support a role of the Wnt signaling pathway in the pathogenesis of PCOS.

The slight increase in postabsorptive EGP associated with FBN3 PCOS risk allele could reflect resistance to insulin-mediated suppression of EGP (33). However, it is also possible that the increase in postabsorptive EGP was due to increased postabsorptive glucose clearance, which in turn could reflect an increase in non-insulin-mediated glucose uptake (33). Although the increase in postabsorptive glucose clearance did not achieve statistical significance in the one-way ANOVA, it was nominally significantly associated with the FBN3 PCOS risk allele in the regression analysis. There may also be alterations in insulin secretion associated with the FBN3 PCOS risk allele since there was a nominally significant decrease in basal insulin secretion rates in this group. Thus, it is possible that a decrease in insulin secretion contributed to an increase in postabsorptive EGP. We failed to confirm the presence of peripheral insulin resistance associated with the FBN3 PCOS risk allele suggested in our earlier study of fasting parameters of insulin resistance (8). Since fasting parameters of insulin resistance also reflect insulin clearance and secretion (34), it is possible that other changes associated with the FBN3 PCOS risk allele accounted for the elevated fasting parameters in that study (8).

Fibrillins are extracellular matrix macromolecules important in connective tissue architecture (35). Fibrillins bind transforming growth factor-β and modulate signaling via this pathway (35). Recent studies support a role for fibrillin-3 in the pathogenesis of PCOS. Examination of fibrillin expression in normal and polycystic ovaries from adult women (36) found significantly decreased fibrillin-3 expression in the perifollicular stroma of follicles in morphological transition from primordial to primary follicles in polycystic ovaries, a stage at which folliculogenesis is disrupted in PCOS (37). Many of the pathways modulated by this signaling family are important in both ovarian function (38) and metabolic processes, including pancreatic islet development, skeletal muscle mass, and adipogenesis (39). Genetic deletion of another extracellular antagonist in the transforming growth factor-β signaling family, follistatin-like 3 gene, whose product also antagonizes activin and myostatin action, in mice produces a metabolic phenotype (40). Accordingly, genetic variation in FBN3 could account for reproductive and metabolic phenotypes in PCOS (8).

In summary, our findings suggest that our previously identified genetic variants in PCOS are associated with metabolic phenotypes. This is particularly true for the association between TCF7L2 PCOS risk allele (rs11196236 G) and M/I in PCOS. However, considering the relatively small sample size of the study and the multigenetic nature of the disorder, these findings require replication in larger independent cohorts.

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**DISCLOSURE**

The authors declared no conflict of interest.

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