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Polycystic Ovary Syndrome is Not Associated with Genetic Variants that Mark Risk for Type 2 Diabetes

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Abstract

Aims/Hypothesis—Polycystic ovary syndrome (PCOS) is a disorder of irregular menses, hyperandrogenism and/or polycystic ovary morphology. A large proportion of women with PCOS also exhibit insulin resistance, β cell dysfunction, impaired glucose tolerance and/or type 2 diabetes (T2D). We therefore hypothesized that genetic variants that predispose to risk for T2D also result in risk for PCOS.

Methods—Variants robustly associated with T2D in candidate gene or genome-wide association studies (GWAS; $n=56$ SNPs from 33 loci) were genotyped in women of European ancestry with PCOS ($n=525$) and controls ($n=472$), aged 18 to 45 years. Metabolic, reproductive and anthropomorphic data were examined as a function of the T2D variants. All genetic association analyses were adjusted for age, BMI and ancestry and were reported after correction for multiple testing.

Results—There was a nominal association between variants in *KCNJ11* and risk for PCOS. However, a risk score of 33 independent T2D-associated variants from GWAS was not significantly associated with PCOS. T2D variants were associated with PCOS phenotype parameters including those in *THADA* and *WFS1* with testosterone levels, *ENPP1/PC1* with triglyceride levels, *FTO* with glucose levels and *KCNJ11* with FSH levels.

Conclusions—Diabetes risk variants are not important risk variants for PCOS.

Clinical Trials Number: NCT00166569

Keywords

testosterone; FSH; hyperandrogenism; triglycerides

Polycystic ovary syndrome is a disorder defined by its cardinal symptoms: anovulation, hyperandrogenism and/or the characteristic polycystic ovary morphology on ultrasound [1]. Insulin resistance, while not a cardinal symptom, is present in a large number of women with PCOS and may be important in the pathogenesis [2, 3]. In addition to insulin resistance, beta cell dysfunction and an increased prevalence of impaired glucose tolerance (IGT) and T2D are demonstrated in women with PCOS compared to BMI-matched controls [4–7]. Conversely, women with T2D have an increased prevalence of polycystic ovary syndrome compared to the general population [8]. There is also a high rate of T2D in family members

of women with PCOS [9]. Therefore, it is possible that genetic variants associated with risk for T2D may also result in risk for PCOS.

We hypothesized that T2D risk variants identified in genome-wide association studies (GWAS), or candidate genes replicated in sufficiently powered studies, also confer risk for PCOS [10–18]. Previous studies investigating the role of T2D genetic variants suggest that they do not contribute strongly to risk of PCOS [19–21]. However, some positive associations have also been reported [22–24]. We therefore sought to test this hypothesis systematically, examining 33 loci identified in previous GWASs or sufficiently powered candidate gene studies of T2D in a cohort of women with PCOS and controls, all well characterized for PCOS-related quantitative traits.

Research Design and Methods

Subjects

All subjects were of European ethnicity, between the ages of 18 and 45 years, and were recruited through advertising from the general population from 2003 to 2011. PCOS subjects were also recruited from reproductive endocrine or primary care outpatient clinics. Subjects with PCOS (n=525) had oligomenorrhea (< 9 menstrual periods/yr) and clinical and/or biochemical evidence of hyperandrogenism, fulfilling the NIH criteria [7]. Control subjects had regular menstrual cycles, 21 to 35 days, and no reported or physical exam evidence of hyperandrogenism (n=472). Subjects were excluded for a personal history or evidence of T2D by fasting or challenge glucose levels [25] and late onset congenital adrenal hyperplasia [7]. All subjects had normal thyroid function and prolactin levels and a follicular phase FSH level in the premenopausal range. Subjects were on no hormonal medication.

Protocol

The study was approved by the Institutional Review Board of the Massachusetts General Hospital and all subjects gave written informed consent. All PCOS subjects were studied 10 days after their last menstrual period and after a 12 hour fast [7]. Subjects underwent a detailed history, physical exam, a pelvic ultrasound (ATL HDI 1500, 5 MHz convex array transducer), fasting blood samples and an oral glucose tolerance test. Hormone assays were performed as previously described [7].

Genotyping

Variants previously demonstrated to confer risk for T2D in GWASs or sufficiently powered candidate gene studies were selected (Supplementary Table 1). Additional variants in the same genes and associated with risk for PCOS in later studies were also genotyped [26, 27], for a total of 60 variants tested. A panel of 152 markers informative for European, African American and Latin ancestry was genotyped to control for false associations related to population stratification [28–30]. 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 genotyping with a sample call rate of <90% and four SNPs failed either design (1 SNP) or quality control (3 SNPs with a call rate of <90%, two of which had an additional departure from Hardy-Weinberg Equilibrium ($p < 1 \times 10^{-3}$) in controls; Supplemental Table 1).

Statistical Analysis

Data that were not normally distributed were log transformed for analysis. Phenotypic characteristics were compared between PCOS cases and controls using a *t* test. PCOS case-control association analysis was performed using a logistic regression framework with SNPs

coded using an additive genetic model. Our 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 T2D risk variants with 30 log-transformed quantitative traits in PCOS cases, controls and the combined sample. Results adjusted for age, BMI and ancestry [28–30] 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 [31]. A genetic risk score of established T2D risk variants (Supplementary Table 1) weighted by the log of the odds ratio from meta-analyses of T2D GWAS was generated as described previously [32]. We evaluated the contribution of the weighted genetic risk score to PCOS in logistic regression models adjusting for age, gender, BMI and 5 principal components.

Results

The anticipated phenotypic differences between PCOS and control subjects were present (Supplementary Table 2). Fifty-six SNPs were successfully genotyped. There were nominal associations between PCOS and variants in *THADA* rs11899863 and rs7578597, *KCNJ11* rs5219 (E23K) and its proxy SNP rs5215, *ZFAND6* rs11634397 and *ENPPI/PC1* rs1044498 and *TCF7L2* PCOS risk variant rs11196229 (Table 1). There were no associations between any of the T2D variants studied and PCOS after correction for multiple testing (Table 1).

Overall, a combined weighted risk score of 29 established independent T2D variants (Supplementary Table 1) was not significantly associated with PCOS (OR 1.03 (0.99–1.07) $p=0.163$).

Quantitative Traits

Six variants were associated with quantitative traits measured in the PCOS and control subjects after controlling for age, BMI, ancestry and multiple testing. The *THADA* rs12468394 A allele was associated with higher testosterone levels, *ENPPI/PC1* rs1044498 C allele with lower triglyceride levels and *CDKAL1* rs7754840 C allele with earlier age at menarche in cases and controls (Table 2). The *KCNJ11* rs5219 (E23K) and rs5215 minor variants were associated with higher FSH levels, *WFS1* rs1801214 C with higher testosterone levels and *FTO* rs9939609 A with higher two hour glucose levels in women with PCOS.

Discussion

Using a PCOS case-control cohort, the data demonstrate that no T2D variants are associated with PCOS risk after adjusting for multiple comparisons. The negative risk score for T2D variants and risk for PCOS further highlights the absence of a relationship between the genetic risk for the two disorders. Further, the variants in *THADA* and *TCF7L2* that were nominally associated with PCOS show an inverse relationship or are different than variants associated with risk for T2D at the same locus, respectively. Thus, the risk variants for PCOS in European women are likely to be unique and related to the cardinal diagnostic criteria.

A nominal association at rs5219 in *KCNJ11* was also demonstrated in a smaller association study in which PCOS subjects were defined as in the current study [19], but not in association studies using a phenotype based on Rotterdam criteria [1, 21, 33] or self reported PCOS symptoms [21]. A weighted z-score based meta-analysis of rs5219 for five European

populations (current study and four previously published results) suggests no association with increased risk of PCOS ($p=0.13$). Interestingly, the *KCNJ11* rs5215 and rs5219 variants were associated with higher FSH levels in PCOS cases, with the highest FSH levels in those subjects carrying the risk allele for diabetes. Perhaps the slightly higher FSH, within the normal range, results in the accelerated follicle development described in previous studies [34, 35]. The variant rs5219 codes for a lysine at position 23 on the inwardly directed potassium channel on the beta cell and is in strong linkage disequilibrium with a functional polymorphism A1369S in the other subunit of the same potassium channel encoded by the neighboring gene *ABCC8* [36]. *KCNJ11/ABCC8* are expressed in the hypothalamus and activated by insulin and leptin [37, 38], suggesting a possible link between the variants and FSH levels in PCOS.

The minor alleles of *THADA* were nominally associated with PCOS, but it is the major alleles that are associated with T2D [11]. While additional variants in *THADA* were associated with risk for PCOS in a GWAS of northern Han Chinese women [27], the PCOS risk variants did not replicate in our European cohort and they were not in linkage disequilibrium with the diabetes risk SNPs ($r^2=0.061$ for rs12478601 and rs7578597 and $r^2=0.048$ for rs12478601 and rs11899863). Paradoxically, we found a strong relationship between the protective allele at rs12468394 and higher testosterone levels. There was no relationship between rs13429458 and testosterone levels in Chinese women, but rs12468394 was not tested [27].

The ectoenzyme nucleotide pyrophosphatase phosphodiesterase 1 (*ENPP1*) or plasma cell membrane glycoprotein-1 (*PC1*) rs1044498 C allele, coding for the 121Q variant, was associated with lower triglyceride levels. *ENPP1* reduces insulin signaling by inhibiting insulin receptor tyrosine kinase activity, and the 121Q variant is a gain of function mutation that results in greater inhibition of insulin stimulated tyrosine kinase activity and greater insulin resistance [39]. Thus, it might be expected that the C allele would be associated with higher triglyceride levels. Previous studies examining triglyceride levels in relation to *ENPP1* genotype have found higher triglyceride levels in subjects carrying at least one Q variant allele [40], or no difference [41]. Of note, the C allele was associated with nominal risk for PCOS, as in a previous small study [42].

We also found a strong relationship between variant rs1801214 in *WFS1* and testosterone levels in women with PCOS. This variant codes for a synonymous amino acid in a base at which a missense change has been described very rarely. *WFS1* is expressed in neurons and β cells of the pancreas and codes for a membrane protein that maintains calcium homeostasis of the endoplasmic reticulum [43]. Interestingly, it is also expressed in the pituitary, hypothalamus and ovary (biogps.gnf.org). Despite the strong relationship with testosterone levels, *WFS1* rs1801214 was not associated with PCOS.

As in most previous studies, *FTO* was not associated with PCOS when corrected for BMI [22, 44]. Nevertheless, even after adjustment for BMI, there was a relationship between *FTO* rs9939609 A and higher 2 hour glucose levels. A similarly increased two-hour glucose level was demonstrated in relation to rs9939609 in women with PCOS in the absence of correction for BMI [45]. However, the relationship between fasting glucose levels and *FTO* is mediated by its effect on BMI in large population studies [46], therefore, larger studies in women with PCOS are needed to determine whether the effect is independent.

The current study is among the largest to examine the relationship between T2D variants and PCOS [19, 22, 26]. It was also the most comprehensive evaluation as our goal was to perform a replication study for a broad range of variants previously identified in GWASs or candidate gene association studies for T2D and PCOS. Therefore, we did not perform a

comprehensive association study for each gene and it is possible that we missed associations between other variants in the genes of interest and PCOS [26]. We had >90% power to detect an association for *TCF7L2* if the OR for risk was similar to that for T2D; i.e. for an OR 1.4 and a MAF of 0.26; 381 subjects were needed. Approximately 1700 cases and controls were needed for 80% power to detect an association between the *KCNJ11* variants and PCOS with an OR 1.14 and minor allele frequency of 0.40, which we were able to achieve with a meta-analysis of existing literature.

The data suggest that there is little overlap in variants that confer risk for T2D and PCOS. Nevertheless, adjusting for these diabetes risk variants in future studies of PCOS genetics may uncover additional risk variants that work through distinct mechanisms unrelated to diabetes risk factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Odds ratios (OR) for PCOS risk at loci identified in GWAS for type 2 diabetes.

Gene	Chr ¹	SNP-allele	P value _{nom} ²	P value _{adj} ³	OR ⁴	#cases	freq-cases	#controls	freq-controls
<i>THADA</i>	2	rs11899863 - T	0.003	0.14	1.80 (1.22–2.65)	518	0.115	467	0.076
<i>THADA</i>	2	rs7578597 - C	0.01	0.49	1.59 (1.10–2.30)	508	0.131	465	0.090
<i>KCNJ11</i>	11	rs5219 - T	0.003	0.15	1.40 (1.11–1.76)	514	0.395	464	0.362
<i>KCNJ11</i>	11	rs5215 - C	0.004	0.16	1.40 (1.11–1.75)	520	0.398	461	0.367
<i>ZFAND6</i>	15	rs11634397 - A	0.007	0.29	0.73 (0.59–0.92)	518	0.323	465	0.369
<i>TCF7L2</i>	10	rs11196229 - A	0.04	0.84	0.76 (0.59–0.99)	518	0.199	464	0.231
<i>ENPP1/PC1</i>	6	rs1044498 - C	0.04	0.86	1.36 (1.01–1.83)	519	0.188	461	0.144
<i>THADA</i>	2	rs12468394 - A	0.05767	0.9382	0.77 (0.58–1.01)	516	0.458	462	0.479
<i>HNF1A</i>	12	rs7957197 - A	0.0832	0.9824	0.79 (0.60–1.03)	521	0.192	447	0.225
<i>DUSP9</i>	23	rs5945326 - G	0.09297	0.989	0.81 (0.64–1.04)	516	0.230	462	0.259
<i>BCL11A</i>	2	rs243021 - A	0.1254	0.9976	1.18 (0.95–1.46)	516	0.479	464	0.519
<i>PPARG</i>	3	rs1801282 - G	0.1302	0.998	0.76 (0.53–1.09)	518	0.0956	457	0.119
<i>HNF1B</i>	17	rs4430796 - G	0.142	0.9988	0.85 (0.68–1.06)	517	0.488	458	0.511
<i>FTO</i>	16	rs11642841 - A	0.1535	0.9992	1.17 (0.94–1.45)	516	0.426	463	0.380
<i>TSPAN8/LGR5</i>	12	rs7961581 - C	0.2132	1	0.86 (0.67–1.09)	503	0.269	435	0.296
<i>HMGGA2</i>	12	rs1531343 - C	0.2148	1	1.23 (0.89–1.70)	517	0.135	460	0.109
<i>KLF14</i>	7	rs972283 - A	0.2288	1	0.88 (0.70–1.09)	511	0.440	463	0.494
<i>MTNR1B</i>	11	rs1387153 - T	0.2324	1	0.87 (0.68–1.10)	516	0.272	460	0.298
<i>TP53INP1</i>	8	rs896854 - T	0.2379	1	0.88 (0.71–1.09)	516	0.472	463	0.499
<i>WFS1</i>	4	rs1801214 - C	0.2555	1	1.14 (0.91–1.42)	515	0.391	457	0.393
<i>TCF7L2</i>	10	rs12255372 - T	0.277	1	.14 (0.90–1.45)	509	0.296	453	0.312
<i>CDKAL1</i>	6	rs10440833 - A	0.2823	1	1.14 (0.89–1.46)	513	0.271	465	0.262
<i>CHCHD9</i>	9	rs13292136 - T	0.283	1	0.78 (0.50–1.22)	517	0.0512	461	0.0632
<i>THADA</i>	2	rs6544661 - A	0.3202	1	1.46 (0.69–3.11)	515	0.423	463	0.435
<i>THADA</i>	2	rs10186921 - C	0.3462	1	1.43 (0.68–3.04)	510	0.452	465	0.457
<i>JAZF1</i>	7	rs849134 - A	0.3486	1	0.90 (0.73–1.12)	517	0.501	463	0.499

Gene	Chr ¹	SNP-allele	P value _{nom} ²	P value _{adj} ³	OR ⁴	#cases	freq-cases	#controls	freq-controls
<i>ZBED3</i>	5	rs4457053 - G	0.3642	1	0.90 (0.71–1.13)	519	0.301	461	0.330
<i>TCF7L2</i>	10	rs11196236 - C	0.3712	1	0.89 (0.68–1.15)	517	0.208	461	0.225
<i>IAZF1</i>	7	rs864745 - T	0.3787	1	0.91 (0.73–1.12)	502	0.493	460	0.494
<i>CDKN2A/B</i>	9	rs10965250 - A	0.3943	1	1.14 (0.85–1.53)	518	0.165	461	0.153
<i>TSPAN8/LGR5</i>	12	rs4760790 - A	0.4119	1	0.90 (0.70–1.15)	517	0.269	464	0.291
<i>CDKAL1</i>	6	rs7754840 - C	0.4531	1	1.10 (0.86–1.39)	521	0.325	463	0.304
<i>THADA</i>	2	rs7567607 - C	0.4773	1	1.17 (0.76–1.79)	516	0.337	456	0.348
<i>SLC30A8</i>	8	rs13266634 - T	0.4852	1	0.92 (0.72–1.16)	518	0.268	464	0.291
<i>CENTD2</i>	11	rs1552224 - C	0.4942	1	1.12 (0.81–1.53)	516	0.140	451	0.137
<i>IRS1</i>	2	rs2943641 - T	0.5165	1	0.93 (0.73–1.17)	518	0.336	465	0.366
<i>THADA</i>	2	rs12478601 - C	0.5281	1	1.26 (0.61–2.63)	517	0.436	462	0.450
<i>THADA</i>	2	rs7582497 - T	0.5761	1	1.13 (0.74–1.72)	517	0.335	462	0.349
<i>KCNQ1</i>	11	rs2237892 - T	0.5857	1	1.14 (0.71–1.83)	517	0.0602	465	0.0480
<i>NOTCH2</i>	1	rs10923931 - T	0.5946	1	1.10 (0.77–1.57)	520	0.107	464	0.0980
<i>IRS1</i>	2	rs7578326 - G	0.5956	1	0.94 (0.75–1.18)	519	0.328	459	0.369
<i>THADA</i>	2	rs6544659 - C	0.5973	1	1.16 (0.67–2.02)	519	0.458	463	0.464
<i>KCNQ1</i>	11	rs231362 - A	0.6332	1	1.05 (0.85–1.31)	513	0.458	465	0.461
<i>ADAMTS9</i>	3	rs4607103 - T	0.6926	1	0.95 (0.73–1.23)	518	0.277	464	0.243
<i>THADA</i>	2	rs6544660 - C	0.7428	1	0.90 (0.49–1.67)	517	0.461	461	0.476
<i>ADAMTS9</i>	3	rs6795735 - T	0.779	1	1.03 (0.83–1.29)	516	0.471	459	0.421
<i>FTO</i>	16	rs9939609 - A	0.8011	1	1.03 (0.83–1.28)	510	0.431	448	0.396
<i>MTNR1B</i>	11	rs10830963 - G	0.8052	1	0.97 (0.76–1.24)	517	0.263	463	0.272
<i>TCF7L2</i>	10	rs7903146 - T	0.8105	1	1.03 (0.81–1.32)	488	0.286	467	0.317
<i>CDKN2A/B</i>	9	rs10811661 - C	0.816	1	1.04 (0.77–1.39)	519	0.162	464	0.156
<i>PPARG</i>	3	rs13081389 - G	0.8518	1	0.95 (0.56–1.62)	518	0.0445	460	0.0525
<i>HNF1B</i>	17	rs757210 - T	0.9332	1	1.01 (0.81–1.26)	518	0.384	466	0.372
<i>THADA</i>	2	rs6751622 - A	0.9516	1	0.98 (0.50–1.94)	523	0.422	465	0.439
<i>THADA</i>	2	rs6746064 - T	0.9596	1	1.02 (0.53–1.97)	516	0.405	463	0.425
<i>PRCI</i>	15	rs8042680 - A	0.9874	1	1.00 (0.80–1.26)	521	0.363	462	0.353

Gene	Chr ¹	SNP-allele	P value _{nom} ²	P value _{adj} ³	OR ⁴	#cases	freq-cases	#controls	freq-controls
HHEX/IDE	10	rs1111875 - T	0.991	1	1.00 (0.81–1.24)	520	0.419	463	0.424

¹Chr - chromosome
²Pvalue_{nom} – nominal p value adjusted for age, BMI and ancestry
³P value_{adj} – p value corrected for multiple comparisons
⁴OR – odds ratio

Table 2

Quantitative Traits Associated Candidate Variants in Women with PCOS or in PCOS Cases plus Controls

Gene	SNP	Group	Allele	Trait	Value	P value
THADA	rs12468394	PCOS & Control	CC	Testosterone (ng/dL)	45.1 ± 2.2	0.001
			CA		49.6 ± 1.6	
			AA		59.8 ± 2.5	
ENPP1/PC1	rs1044498	PCOS & Control	AA	Triglycerides (mg/dL)	88.9 ± 2.3	0.04
			CA		74.1 ± 3.7	
			CC		78.9 ± 15.8	
CDKAL1	rs7754840	PCOS & Controls	GG	Age at Menarche	12.75 ± 0.07	<0.01
			GC		12.50 ± 0.07	
			CC		12.32 ± 0.16	
KCNJ11	rs5219	PCOS	CC	FSH (IU/L)	9.8 ± 0.3	<0.05
			CT		10.8 ± 0.3	
			TT		11.1 ± 0.5	
KCNJ11	rs5215	PCOS	TT	FSH (IU/L)	9.8 ± 0.3	<0.05
			TC		10.8 ± 0.3	
			CC		11.0 ± 0.5	
WFS1	rs1801214	PCOS	CC	Testosterone (ng/dL)	72.4 ± 4.1	0.003
			CT		68.4 ± 2.4	
			TT		55.9 ± 2.8	
FTO	rs9939609	PCOS	TT	Glucose 120 PCOS	94.4 ± 2.7	0.03
			TA		104.1 ± 2.1	
			AA		110.5 ± 3.6	