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A Variant in the Fibrillin-3 Gene is Associated with TGF-β and Inhibin B Levels in Women with Polycystic Ovary Syndrome

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Abstract

In an attempt to evaluate the association between Allele 8 (A8) of D19S884 in the fibrillin-3 gene and circulating TGF-β and inhibin levels in women with polycystic ovary syndrome (PCOS), we studied 120 similarly aged women from families with PCOS and compared 40 women with PCOS who did not have A8 (A8− PCOS) to 40 women with PCOS who had A8 (A8+ PCOS) and 40 normally menstruating women who did not have either PCOS or A8 (A8− Non-PCOS). A8−PCOS is associated with higher levels of TGF-β1 compared to A8+ PCOS or A8− Non-PCOS, similar levels of TGF-β2 compared to A8+ PCOS but lower levels of TGF-β2 compared to A8− Non-PCOS, and lower levels of Inhibin B and aldosterone compared to A8+ PCOS.

Key terms

polycystic ovary syndrome; TGF-β; inhibin; fibrillin; genetic association study; hyperandrogenism; insulin resistance

Recent genetic studies have reported that Allele 8 (A8) of D19S884, a dinucleotide repeat polymorphism in intron 55 of the fibrillin-3 gene, is linked to polycystic ovary syndrome

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(PCOS) and associated with insulin resistance in women with PCOS (1–4). The fibrillins are extracellular matrix proteins that regulate the activity of the TGF-β superfamily, multifunctional cytokines involved in cell proliferation, differentiation, and apoptosis (5). TGF-β dysregulation due to mutations in fibrillin-1 can contribute to cardiovascular and other connective tissue abnormalities found in Marfan syndrome (6,7). Similarly, TGF-β dysregulation due to variants in fibrillin-3 could contribute to the reproductive and cardiometabolic dysfunction observed in PCOS.

Evidence that TGF-β dysregulation contributes to PCOS comes from studies demonstrating reproductive dysfunction in knockout mice at all levels of this important signaling pathway (8). Although the roles of TGF-β1 and TGF-β2 in PCOS have not been investigated, other members of the TGF-β superfamily, including activins, inhibins, and Anti-Müllerian hormone (AMH) have been implicated in the pathogenesis of PCOS (9–11). Dysregulation of TGF-β may also contribute to cardiometabolic aspects of PCOS through increased renin-angiotensin-aldosterone system activity, insulin resistance, inflammation, and subclinical atherosclerosis (12–15).

The primary aim of our study was to test the hypothesis that the presence of A8 would be associated with alterations in circulating TGF-β, inhibin, and aldosterone levels in women with PCOS.

The Institutional Review Board of the Pennsylvania State University College of Medicine approved the study. Written informed consent was obtained from all subjects. A total of 120 similarly aged women were studied, including 40 with PCOS who have A8 (A8+ PCOS), 40 with PCOS who do not have A8 (A8− PCOS), and 40 Control women without either PCOS or A8 (A8− Non-PCOS). Subjects were recruited by physician referral, patient referral, and response to advertisements for an ongoing PCOS genetics study. All controls were unaffected sisters of women with PCOS. Three controls had sisters in the A8+ PCOS group and eight had sisters in the A8− PCOS group. The remaining controls were sisters of PCOS women but their PCOS sisters were not included in the study. We previously reported phenotype and genotype information on the majority of women in the study (1). This study commenced before publication of the Rotterdam criteria (16), so PCOS was defined as unexplained hyperandrogenic chronic anovulation according to the National Institutes of Health criteria (17). Therefore, ovarian ultrasound morphology information was not available in many subjects and was not needed to make the diagnosis (18,19). Secondary causes of hyperandrogenemia and anovulation were excluded (19,20). Non-PCOS women were defined as having normal androgen levels and regular menses every 27–35 days (19). None of the PCOS or control subjects were using medications known to affect reproductive hormones or insulin sensitivity, including oral contraceptives and metformin.

Anthropometric measurements were taken as previously reported (19). Acne scores were recorded. The presence and severity of hirsutism was assessed using the modified Ferriman-Gallwey score (21). Morning fasting blood samples were obtained from all PCOS and control subjects. The controls were encouraged to have their blood drawn during the follicular phase of their menstrual cycle, although this was sometimes not possible. Blood samples were obtained randomly in those with oligomenorrhea. Circulating total (active + latent) TGF-β1 and TGF-β2 were measured using Quantikine ELISA kits (R&D Systems, Minneapolis, Minnesota). Inhibin A, Inhibin B, and AMH were measured by ELISA using Diagnostic Systems Laboratories reagents (Beckman Coulter, Webster, Texas). Aldosterone was measured by RIA using reagents obtained from Siemens Corporation (Los Angeles, CA). High sensitive C-reactive protein (hsCRP) was determined by ELISA using reagents obtained from ALPCO Diagnostics (Salem, NH). Androgens, glucose, insulin, and lipid profiles were determined as previously described (15,22,23).

Genotyping of D19S884 was performed as previously described (18). Even though multiple alleles have been found at the D19S884 locus, only allele 8 (A8) has been linked to PCOS. Therefore, subjects were classified as A8+ if they possessed one or two A8 alleles and A8− if they did not. Among the 40 A8+ PCOS women, 4 women were homozygous for A8, having inherited A8 from both their mother and father. We re-analyzed the data after excluding the 4 women homozygous for A8 and found similar results. Therefore, we decided not to exclude the 4 homozygous women in our report. The number of homozygous women was too few to examine allele dose effects.

Linear mixed effects models were fit to continuous outcomes, such as TGF- β1 and TGF-β2, to compare groups. The mixed effects models consisted of a fixed effect for group (A8+ PCOS, A8− PCOS, and A8− Non-PCOS), a random effect for family to account for familial relationships of subjects, and the adjustment covariate of body mass index (BMI). The effect size from these models was quantified using model-adjusted means and 95% confidence intervals (CI). The Bonferroni procedure was used to adjust values and 95% CIs to account for multiple testing per outcome. All hypothesis tests were two-sided and all analyses were performed using SAS software, version 9.1 (SAS Institute Inc., Cary, NC).

The clinical and biochemical characteristics of the 120 women are shown in Table 1 according to PCOS and A8 status. Compared with A8− PCOS, A8+ PCOS women had significantly decreased levels of TGF-β1. Compared with A8− Non-PCOS, A8− PCOS patients had significantly increased TGF-β1 levels. TGF-β2 was similar in the A8+ and A8− PCOS groups, but significantly reduced compared to the Non-PCOS group. Among women with PCOS the presence of A8 was associated with significantly greater Inhibin B levels. Inhibin A levels were similar in the A8+ PCOS and A8− PCOS groups, but significantly reduced compared to the Non-PCOS group. AMH levels, T levels, Ferriman-Gallwey hirsutism scores and acne scores were similar in the A8+ PCOS and A8− PCOS groups, but significantly increased compared to the A8− Non-PCOS group.

Aldosterone levels were significantly higher in the A8+ PCOS women compared to A8− PCOS women, despite similar blood pressure and potassium levels. Aldosterone levels were similar in the A8− PCOS and A8−Non-PCOS groups. There were no statistically significant differences in fasting glucose, insulin, HOMA-IR, hsCRP, blood pressure and lipids when comparing A8+ PCOS to A8− PCOS and A8− PCOS to A8− Non-PCOS. We did not analyze differences between A8+PCOS vs. A8−Non-PCOS because would be impossible to determine whether such differences were due to the presence of A8 or PCOS.

The present study is the first to demonstrate that women with PCOS have increased TGF-β1 levels, unless they are A8+ in which case their TGF-β1 levels are comparable to A8−Non-PCOS women. The increase in TGF-β1 levels in A8−PCOS could be due to other aspects of PCOS such as variations in follistatin. Follistatin regulates activin and other members of the TGF-β superfamily in a manner identical to the regulation of TGF-β by fibrillins (24,25). The role of follistatin in PCOS is unclear. The follistatin gene was linked to PCOS in an early genetic study, but a subsequent study looking at allelic variants of the follistatin gene concluded that the contributions of follistatin to PCOS were likely to be small (18,26). We did not measure follistatin in our study, however increased circulating follistatin levels have been reported in women with PCOS (11). The lack of an increase in TGF-β1 levels in A8+PCOS suggests that the presence of A8 overrides the other effects of PCOS on TGF-β1. PCOS itself does not alter Inhibin B levels, unless A8 is present in which case Inhibin B levels are increased and could contribute to reproductive dysfunction. These findings support our overall conclusions regarding the potential importance of A8 and the TGF-β pathway in PCOS.

Our findings disagree with a recent study by Prodoehl and colleagues which demonstrated relatively low ovarian fibrillin-3 expression and no association between A8 or SNPs in the fibrillin-3 gene and PCOS (27). Based on their findings Prodoehl and colleagues concluded that the A8 variant of fibrillin-3 was not involved with PCOS, but they conceded that they could not exclude the possibility that fibrillin-3 expression in other tissues alter the PCOS phenotype. Supporting the latter, we found that the presence of A8 is clearly associated with alterations in circulating TGF-β and Inhibin B levels among women with PCOS. A potential explanation for the discrepancy between these two studies is the genetic and environmental differences between the two study populations.

Including a fourth group of A8+ non-PCOS women would have allowed us to determine if A8 contributes to TGF-β dysregulation in non-PCOS women just as it does in PCOS. However this was not possible because the number of A8+ non-PCOS sisters in our genetics study were too few to allow for any meaningful comparisons.

Our findings support the hypothesis that the A8 variant of fibrillin-3 is associated with dysregulation of TGF-β and may contribute to the pathogenesis of reproductive and cardiometabolic abnormalities in women with PCOS. Future studies are needed to confirm these findings and to determine the extent to which variants in other fibrillins, fibrillin-like proteins such as follistatin, or other components of the TGF-β signaling pathway contribute to PCOS.

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Table 1

Clinical and biochemical characteristics in A8+ PCOS, A8− PCOS and A8− Non-PCOS women.[a]

	A8+ PCOS Model-adjusted Mean (SD) [n]	A8− PCOS Model-adjusted Mean (SD) [n]	A8− Non-PCOS Model-adjusted Mean (SD) [n]	A8+ PCOS vs. A8− PCOS P-value[b]	A8− PCOS vs. A8− Non-PCOS P-value[b]
Biometrics					
Age (years)	27.7 (1.6) [40]	27.1 (0.7) [40]	28.1 (9.0) [40]	1.00	1.00
BMI (kg/m^2)	37.1 (8.2) [40]	33.2 (8.0) [40]	27.7 (6.1) [40]	0.14	0.01
SBP (mm Hg)	119.2 (14.4) [39]	117.6 (10.8) [34]	116.8 (8.2) [37]	1.00	1.00
DBP (mmHg)	75.7 (8.0) [39]	74.7 (7.6) [34]	71.8 (6.9) [37]	1.00	0.42
TGF-β Superfamily					
TGF-β1 (ng/ml)	26.9 (7.1) [40]	37.2 (12.5) [40]	28.8 (5.5) [40]	0.003	0.008
TGF- β2 (pg/ml)	188.3 (48.3) [40]	216.2 (117.6) [40]	314.8 (100.0) [40]	0.63	0.01
Inhibin A (pg/ml)[c]	3.6 (1.4, 8.1) [40]	1.5 (0.8, 2.4) [40]	11.9 (3.6, 23.8) [40]	0.11	<0.001
Inhibin B (pg/ml)[c]	49.7 (20.1, 76.1) [40]	17.7 (7.9, 35.6) [40]	25.5 (7.2, 61.0) [40]	0.006	1.00
AMH (ng/ml)	7.9 (3.5) [40]	9.1 (5.2) [40]	3.0 (2.4) [40]	0.56	<0.001
Reproductive profile					
Total testosterone (ng/dl)	79.6 (28.0) [40]	77.7 (19.0) [40]	31.0 (12.0) [40]	1.00	< 0.001
Unbound testosterone (ng/dl)	25.0 (9.0) [40]	23.4 (10.1) [40]	6.4 (3.4) [40]	0.93	< 0.001
SHBG (nmol/L)	77.0 (19.0)	84.9 (22.0)	109.7 (69.2)	0.81	0.20

	A8+ PCOS Model-adjusted Mean (SD) [n]	A8− PCOS Model-adjusted Mean (SD) [n]	A8− Non-PCOS Model-adjusted Mean (SD) [n]	A8+ PCOS vs. A8− PCOS P-value[b]	A8− PCOS vs. A8− Non-PCOS P-value[b]
	[36]	[34]	[37]		
Ferriman-Gallwey	11.0 (7.1)	14.7 (7.1)	6.6 (4.9)	0.10	<0.001
hirsutism score	[37]	[39]	[33]		
Metabolic profile					
Aldosterone (ng/dl)	11.4 (6.8)	7.6 (4.0)	8.1 (5.5)	0.04	1.00
	[40]	[40]	[40]		
Glucose (mg/dl)[d]	89.6 (11.1)	86.2 (8.6)	87.4 (7.8)	0.33	1.00
	[40]	[40]	[40]		
Insulin (μU/ml)[e]	25.4 (13.3)	21.4 (11.0)	21.5 (9.2)	0.34	1.00
	[40]	[40]	[40]		
HOMA-IR	3.2 (1.6)	2.7 (1.3)	2.7 (1.1)	0.29	1.00
	[40]	[40]	[40]		
LDL (mg/dl)	117.4 (29.4)	113.3 (31.3)	115.2 (32.2)	1.00	1.00
	[36]	[39]	[37]		
Triglycerides (mg/dl)	135.5 (82.2)	156.0 (65.9)	128.0 (25.8)	0.63	0.16
	[37]	[40]	[37]		

[a] Data are based on ANCOVA model adjusting for BMI, except for age and BMI which are based on ANOVA (unadjusted).

[b] Bonferroni adjustment for multiple tests.

[c] Data reported as median (25^{th} percentile, 75^{th} percentile) adjusted for BMI due to skewness.

[d] To convert glucose to millimoles per liter, multiply by 0.05551.

[e] To convert insulin to picomoles per liter, multiply by 6.945.