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Variation in the perilipin gene (*PLIN*) affects glucose and lipid metabolism in non-Hispanic white women with and without polycystic ovary syndrome

Toshihide Kawai [a,*], *Maggie C.Y. Ng* [a,d], *M. Geoffrey Hayes* [a], *Issei Yoshiuchi* [a], *Takafumi Tsuchiya* [a], *Heather Robertson* [c], *Nancy J. Cox* [a,b], *Kenneth S. Polonsky* [c], *Graeme I. Bell* [a,b], *David A. Ehrmann* [a]

[a] *Department of Medicine, The University of Chicago, Chicago, IL, USA*
[b] *Department of Human Genetics, The University of Chicago, Chicago, IL, USA*
[c] *Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA*
[d] *Department of Pediatrics, Section on Medical Genetics, and Centers for Human Genomics and Diabetes Research, Wake Forest University School of Medicine, Winston-Salem, NC, USA*

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ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women. It is characterized by chronic anovulation, hyperandrogenism, obesity and a predisposition to type 2 diabetes mellitus (T2DM). Since obesity plays an important role in the etiology of PCOS, we sought to determine if variants in the perilipin gene (*PLIN*), a gene previously implicated in the development of obesity, were also associated with PCOS. We typed six single nucleotide polymorphisms (haplotype tagging and/or previously associated with obesity or related metabolic traits) in *PLIN* in 305 unrelated non-Hispanic white women (185 with PCOS and 120 without PCOS). None of the variants was associated with PCOS ($P < 0.05$). However, the variant rs1052700*A was associated with increased risk for glucose intolerance (impaired glucose tolerance or T2DM) in both non-PCOS (OR = 1.75 [1.02–3.01], $P = 0.044$) and PCOS subjects (OR = 1.67 [1.08–2.59], $P = 0.022$). It was also associated with increased LDL ($P = 0.007$) and total cholesterol levels ($P = 0.042$). These results suggest that genetic variation in *PLIN* may affect glucose and lipid metabolism in women both with and without PCOS.

1. Introduction

The clinical manifestations of polycystic ovary syndrome (PCOS), one of the most common endocrine disorders in women include chronic anovulation, hyperandrogenism, obesity, and a predisposition to type 2 diabetes mellitus (T2DM) [1–4]. Insulin resistance is a key contributor to the phenotypic manifestations of PCOS and appears to be a heritable component of the disorder [4–7]. Thus, efforts to identify genes contributing to the etiology of PCOS have focused on those affecting metabolic pathways related to obesity and insulin action [8–10].

Perilipins are hormonally regulated phosphoproteins found on the surface of lipid storage droplets in adipocytes and steroidogenic cells of the adrenal cortex, testes and ovaries [11]. They modulate deposition and mobilization of triglycerides in the adipocyte by inhibiting hormone sensitive lipase (HSL)-mediated lipolysis [12,13]. Targeted knockout of the perilipin gene in mice results in constitutive activation of adipocyte HSL and resistance to diet-induced and genetic obesity [12,13].

* Corresponding author at: Department of Medicine, The University of Chicago, 5841 S. Maryland Ave., MC1027, Chicago, IL 60637, USA. Tel.: +1 773 702 9116; fax: +1 773 702 9237.
E-mail address: tkawai@sc.itc.keio.ac.jp (T. Kawai).
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Variants in the perilipin gene (*PLIN*) has been previously associated with measures of obesity and lipid levels in humans [14–23]. We therefore hypothesized that variants in *PLIN* may be associated with the phenotype of PCOS. To test this hypothesis, we examined variants in *PLIN* for association with PCOS as well as metabolic abnormalities characteristic of PCOS.

2. Research design and methods

Non-Hispanic white women with PCOS and without PCOS living in Chicago and St. Louis were recruited for this study. Subjects with PCOS were recruited without regard to personal or family history of glucose tolerance. All were at least 2 years post-menarche and <40 years of age. A diagnosis of PCOS required the presence of oligo/amenorrhea, hyperandrogenemia (plasma free testosterone level ≥34.7 pmol/L), hyperandrogenism as evidenced by infertility, hirsutism, acne or androgenetic alopecia, and exclusion of nonclassic 21-hydroxylase deficiency congenital adrenal hyperplasia, hypothyroidism, or significant elevations in serum prolactin (when screened for clinically indicated Cushing's syndrome). The non-PCOS group was comprised of healthy post-pubertal girls and women. The study was approved by the Institutional Review Boards of the University of Chicago and Washington University, St. Louis and written informed consent was obtained from each subject.

All individuals, with the exception of those with known T2DM, had an oral glucose tolerance test (OGTT). After an overnight fast, blood samples were obtained at times −15 and 0 min. 75 g of dextrose was then administered orally and blood samples were obtained at 30, 60, 90 and 120 min for measurement of glucose and insulin concentrations. Glucose tolerance status was based upon the plasma glucose concentration at 2 h using criteria of the American Diabetes Association [24]. A diagnosis of normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or T2DM was assigned if the glucose level at 2 h was <7.8 mmol/L, between 7.8 and 11.1 mmol/L, or >11.1 mmol/L, respectively.

Plasma glucose was measured immediately using an automated glucose analyzer (YSI Model 2300 STAT, Yellow Springs Instruments Co., Yellow Springs, OH). Serum insulin was measured using a double-antibody technique in the Ligand Assay Core laboratories of the University of Chicago and Washington University Diabetes Research and Training Centers.

Genomic DNA was isolated from peripheral blood lymphocytes. We genotyped seven single nucleotide polymorphisms (SNPs) (rs4578621, rs2289487, rs6496589, rs894160, rs894162, rs2304795 and rs1052700) using Taqman-based assays on an ABI Prism 7700 Sequence Detector (Applied Biosystems, Foster City, CA); however, the assay for rs894160 failed. These seven SNPs were selected because they tagged ($r^2 = 0.8$) all SNPs within 2 kb upstream and downstream of *PLIN* with minor allele frequency (MAF) > 0.01 in the HapMap phase II Utah residents with ancestry from northern and western Europe (CEU), or showed association with obesity or metabolic traits in previous studies [14–23]. All polymorphisms (e.g. rs4578621 C>T) are labeled by their dbSNP rs# with the common allele followed by the rare allele in the minus strand orientation (forward transcription orientation).

Hardy–Weinberg equilibrium (HWE) was assessed for each SNP in PCOS and non-PCOS groups separately. Estimated pairwise linkage disequilibrium (LD) was performed using JLIN (http://www.genepi.com.au/projects/jlin; [25]). Allele frequencies differences between PCOS and non-PCOS groups were compared using a χ^2 test or Fisher's exact test as appropriate and presented with odds ratio (OR) and 95% confidence interval (CI). We compared the haplotype frequency distribution in subjects with and without PCOS using PHASE (v. 2.0.2, http://www.stat.washington.edu/stephens/software.html [26,27]. Multivariate linear regression was used to assess the association between genetic variants at *PLIN* and phenotypic traits with or without adjustment for age and BMI as covariates. Subjects with impaired glucose tolerance or T2DM were removed from consideration in this portion of the analysis as their values for many of the metabolic phenotypes could be affected by hyperglycemia and/or hyperinsulinemia. Continuous variables that were not normally distributed were logarithmically transformed and expressed as mean ± standard deviation (SD) or geometric mean (95% CI). Additive genetic models were used except for rs4578621 in the non-PCOS group in which case a dominant genetic model for the minor allele was used due to the small number ($N = 2$) of individuals homozygous for the minor allele. All statistical tests were performed with SPSS (SPSS for Windows, v. 11.5; SPSS, Chicago, IL, USA) unless specified otherwise.

Table 1 – Clinical characteristics of non-Hispanic white female PCOS and non-PCOS subjects classified by glucose tolerance status.

	NGT			IGT and T2DM		
	Non-PCOS	PCOS	*P*	Non-PCOS	PCOS	*P*
N	77	102		43	83	
Age (yr)	32.3 ± 10.9	28.4 ± 6.1	0.005	41.6 ± 10.6	30.8 ± 6.3	<0.001
Body mass index (kg/m^2)	26.8 ± 6.8	36.3 ± 8.0	<0.001	31.6 ± 8.4	38.2 ± 7.3	<0.001
Glycohemoglobin (%)	5.3 (5.2–5.4)	5.3 (5.1–5.4)	0.676	5.6 (5.5–5.8)	5.5 (5.3–5.7)	0.298
Fasting glucose (mmol/L)	5.0 (4.9–5.0)	5.0 (4.9–5.1)	0.792	5.3 (5.0–5.5)	5.4 (5.3–5.6)	0.269
2-h glucose (mmol/L)	6.5 (6.4–6.7)	6.3 (6.2–6.5)	0.151	9.2 (8.8–9.6)	9.3 (9.0–9.6)	0.577
Fasting insulin (pmol/L)	30.2 (25.6–35.5)	120.2 (105.2–137.3)	<0.001	54.6 (42.6–70)	162.0 (138.0–190.2)	<0.001
2-h insulin (pmol/L)	197.5 (174.3–223.8)	603.4 (525.5–692.9)	<0.001	406.5 (336.6–490.8)	1186.2 (1034–1360.7)	<0.001
HOMA-IR	1.1 (0.9–1.3)	4.4 (3.8–5.1)	<0.001	2.1 (1.6–2.8)	6.5 (5.5–7.7)	<0.001

Mean ± SD or geometric mean (95% CI) are shown.