Variation in the Perilipin Gene (*PLIN*) Affects Glucose and Lipid Metabolism in Non-Hispanic White Women With and Without Polycystic Ovary Syndrome

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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.

Keywords

Perilipin; polycystic ovary syndrome (PCOS); type 2 diabetes mellitus; glucose; lipid

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DECLARATION OF INTEREST

The authors report no conflict of interest.

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]. 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.

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 $\geq$ 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.

RESULTS

We studied 185 and 120 women with and without PCOS respectively. All were of European descent and classified as non-Hispanic white. Compared to the non-PCOS group, those with PCOS were significantly younger (Table 1). Within the PCOS group, 102 subjects (55%) had NGT, 72 (39%) had IGT and 11 (6%) had T2DM, based on the results of an OGTT. In contrast, 77 (64%) of non-PCOS women had NGT, 40 (33%) had IGT and 3 (3%) had T2DM.

We studied six single nucleotide polymorphisms (SNPs) including two coding and four non-coding SNPs that were successfully genotyped (Figure 1). All SNPs were in Hardy-Weinberg equilibrium in both PCOS and non-PCOS groups. These SNPs covered 85% of HapMap phase II SNPs in this region at r^2 of 0.8 in the CEU samples, and/or were associated with metabolic or obesity related phenotypes in previous studies [14-23]. There were no significant differences in allele frequencies between PCOS and non-PCOS subjects (Figure 1) although the minor C allele of rs2304795 (rs2304795*C) approached but did not reach statistical significance with PCOS (OR = 1.39 (95% CI = 0.99-1.96), *P* = 0.059). PCOS and non-PCOS groups had significantly different six-SNP haplotype frequencies (*P* = 0.04), but after the two rare polymorphisms (rs6496589 and rs894162; minor allele frequency (MAF) < 0.02) were removed from consideration, the four-SNP haplotype frequencies were no longer significantly different (*P* = 0.08). These two SNPs were not included in subsequent analyses.

Since glucose intolerance (IGT and T2DM) is common in PCOS, we tested the hypothesis that genetic variation in *PLIN* may modulate the glucose and insulin responses to an oral glucose challenge in these subjects. The rs1052700*A allele was associated with glucose intolerance in PCOS (OR = 1.67 (1.08-2.59), *P* = 0.022; Table 2). Similar effects were observed in subjects without PCOS (OR = 1.75 (1.02-3.01), *P* = 0.044). The rs4578621*G allele was also associated with glucose intolerance in PCOS subjects (OR = 2.61 (1.05-6.46), *P* = 0.039) but not in non-PCOS subjects (OR = 1.17 (0.48-2.82), *P* = 0.729). The associations observed with these two

SNPs represent independent observations as there is no substantial linkage disequilibrium (LD) between them (D' = 0.52, r^2 = 0.05, Figure 1).

The effect of *PLIN* upon metabolic measures in normoglycemic non-PCOS subjects were assessed by examining the genotypic associations with BMI, lipids, glucose and insulin levels obtained during the OGTT (Table 3). The variant rs1052700*A was associated with low density lipoprotein cholesterol (LDL-C) and total cholesterol levels even after adjustment for age and BMI in the case of LDL-C (P = 0.007 and P_{adj} = 0.014 for LDL-C; P = 0.042 and P_{adj} = 0.073 for total cholesterol). The variant rs2289487*G was also associated with LDL-C level (P = 0.036 and P_{adj} = 0.046) and fasting glucose level (P = 0.045 and P_{adj} = 0.039) in normoglycemic non-PCOS subjects. However, none of the metabolic phenotypes including BMI, sex hormone concentrations, glucose and insulin levels at basal and during OGTT showed significant association with *PLIN* in the normoglycemic PCOS women (data not shown).

DISCUSSION

Our results are consistent with earlier studies indicating that variants in the *PLIN* gene are associated with metabolic abnormalities related to obesity, insulin resistance and glucose intolerance in women [14-23]. Our results further suggest that genetic variation in *PLIN* is associated with glucose intolerance in non-Hispanic white women. These effects, particularly for the variant rs1052700, were seen in women both with and without PCOS suggesting they are not related to PCOS *per se*.

The molecular mechanism(s) by which perilipins affect glucose and lipid metabolism *in vivo* remain unclear. Perilipins increase triacylglycerol storage in adipocytes by forming a physical barrier that reduces the action of soluble lipases on stored lipids, thus inhibiting triacylglycerol hydrolysis in the basal state [12,13,28]. Phosphorylation of perilpins allows HSL to hydrolyze triacylglycerol [12,13,29]. In addition, tumor necrosis factor–α, one of the key adipokines associated with insulin resistance, has been reported to affect perilipin function [30]. Thus, it seems reasonable to assume that perilipin might affect glucose intolerance via the modulation of lipid metabolism. We also observed that rs2289487*G and rs1052700*A are associated with LDL-C levels. A previous study showed that two other SNPs in *PLIN*, rs2289487 and rs894160, were associated with LDL-C levels [15]. The variant rs2289487 is in moderate LD with rs1052700 (r^2 = 0.349) and rs894160 (r^2=0.733) in the CEU HapMap sample suggesting that the SNP cluster rs1052700, rs894160 and rs2289487 are associated with LDL-C levels.

In summary, our studies suggest that genetic variation in *PLIN* affects glucose and lipid metabolism in women and perhaps risk of PCOS. However, our sample size is small and as a consequence, it is important to determine if these results can be replicated in studies of larger groups of women, both non-Hispanic white and other racial groups.

Acknowledgments

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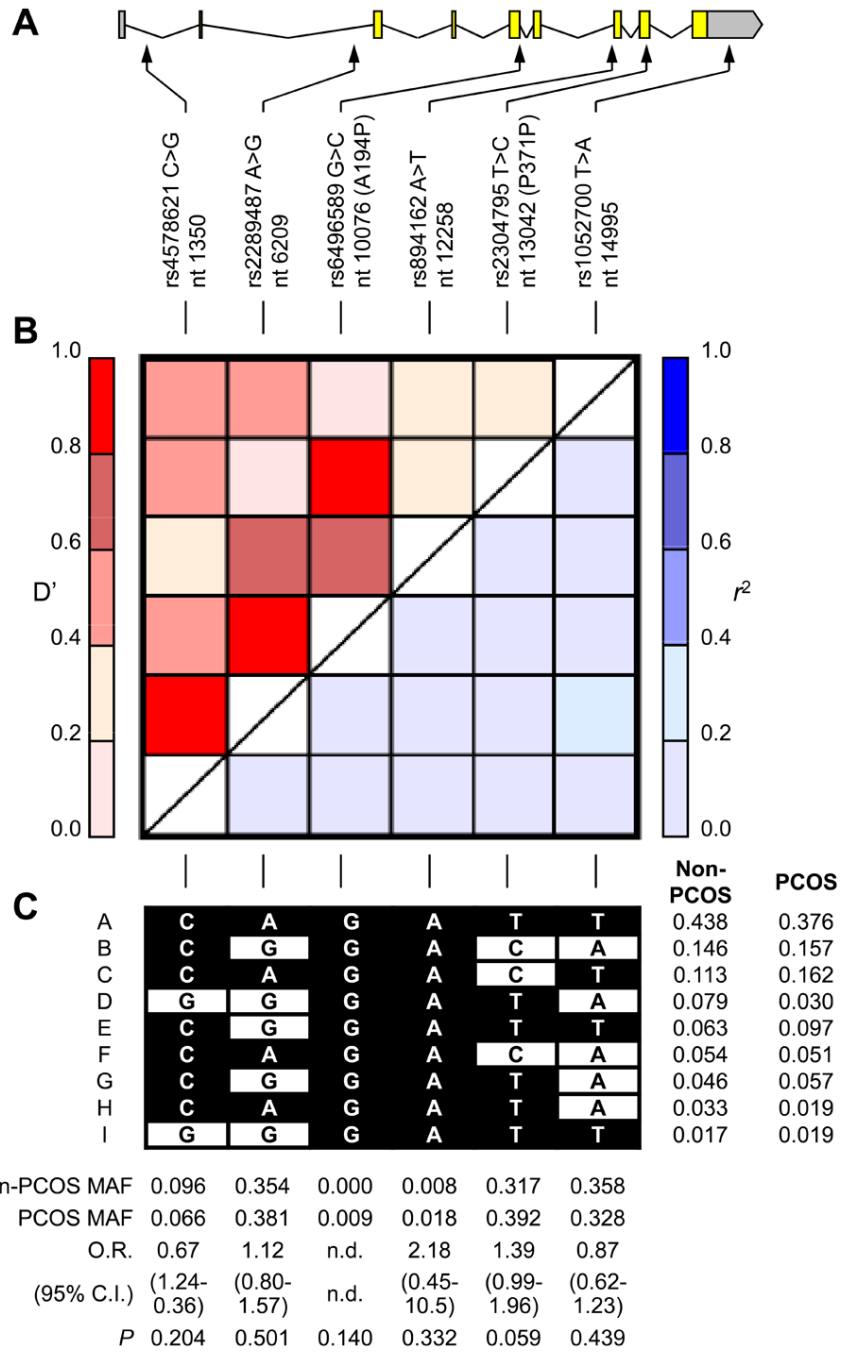
Fig. 1.
PLIN structure, linkage disequilibrium, and genotype and haplotype frequencies. *A*: Intron/exon structure of *PLIN* and the location of SNPs studied. The sequence and intron/exon boundary of *PLIN* was based on GenBank accession number AB005293. rs4578621 C>T, etc., denote the polymorphism and refer to the common and rare alleles, respectively. Nucleotide (nt) positions are indicated (the "A" of the initiator Methionine ATG codon at NT_010274 position 5188832 is nt position 1), and synonymous and nonsynonymous coding region polymorphisms follow in brackets. *B*: Pairwise linkage disequilibrium of *PLIN* SNPs. D' is shown in upper left triangle and r^2 in lower right triangle. *C*: Common haplotypes. Haplotypes occurring at frequency > 0.01 are shown with their frequencies in PCOS and non-PCOS groups

on the right. Common alleles are indicated by black squares, and rare alleles in white squares. Beneath each SNP is the minor allele frequency (MAF) in the PCOS and non-PCOS groups and the results of allelic association tests.

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.

Table 2

Association of *PLIN* polymorphisms with glucose tolerance in non-Hispanic white women

Comparison	N	rs4578621 C/G [*]			rs2289487 A/G [*]			rs2304795 T/C [*]			rs1052700 T/A [*]		
		MAF	OR[†] (95% CI)	P	MAF	OR[†] (95% CI)	P	MAF	OR[†] (95% CI)	P	MAF	OR[†] (95% CI)	P
non-PCOS NGT vs.	77	0.091	1		0.318	1		0.299	1		0.312	1	
non-PCOS IGT/T2DM	43	0.105	1.17 (0.48–2.82)	0.729	0.419	1.54 (0.89–2.66)	0.119	0.349	1.26 (0.72–2.20)	0.423	0.442	1.75 (1.02–3.01)	0.044
PCOS NGT vs.	102	0.040	1		0.358	1		0.363	1		0.277	1	
PCOS IGT/T2DM	83	0.099	2.61 (1.05–6.46)	0.039	0.410	1.25 (0.82–1.90)	0.308	0.428	1.31 (0.86–2.00)	0.203	0.390	1.67 (1.08–2.59)	0.022

MAF, minor allele frequency

[*] The first and second alleles represent the major and minor alleles, respectively.

[†] for minor allele as risk allele.

Table 3

Association of *PLIN* polymorphisms with metabolic traits in normoglycemic non-Hispanic white women without PCOS

rs2289487

	AA	AG	GG	P*	P_adj †
N	36	33	8		
Age (yr)	33.1 ± 11.6	32.2 ± 10.6	28.8 ± 8.9	0.363	
BMI (kg/m²)	26 ± 5.7	26.8 ± 6.3	30.8 ± 11.8	0.117	
Total cholesterol (mg/dL)	159.7 ± 23.7	174 ± 31.8	162.4 ± 26.2	0.262	0.276
Triglycerides (mg/dL)	64.5 (48.7-85.3)	77 (63.3-93.7)	45.6 (22-94.5)	0.832	0.543
HDL (mg/dL)	56.6 ± 12.5	52.9 ± 11.4	50.6 ± 12.5	0.186	0.383
LDL (mg/dL)	86.9 ± 20.3	103.7 ± 27.6	101.2 ± 26	**0.036**	**0.046**
Glycohemoglobin (%)	5.3 (5.1-5.4)	5.3 (5.2-5.4)	5.4 (5.1-5.7)	0.263	0.317
Fasting glucose (mmol/l)	4.9 (4.8-4.9)	5 (4.9-5.2)	5 (4.7-5.4)	**0.045**	**0.039**
2-h glucose (mmol/l)	6.5 (6.2-6.8)	6.5 (6.3-6.8)	6.8 (6.2-7.4)	0.511	0.438
Fasting insulin (pmol/l)	26.9 (21.5-33.8)	30.8 (24-39.6)	46.3 (21.5-99.4)	0.071	0.537
2-h insulin (pmol/l)	189.1 (160.4-222.9)	200.3 (162.1-247.4)	227.5 (129.1-401)	0.398	0.923
HOMA-IR	1 (0.8-1.2)	1.1 (0.9-1.5)	1.7 (0.8-3.9)	0.053	0.418

rs1052700

	TT	TA	AA	P*	P_adj †
N	36	34	7		
Age (yr)	31.4 ± 10.8	33.7 ± 11.2	29.6 ± 10.8	0.856	
BMI (kg/m²)	25.6 ± 6	28.2 ± 7.9	26.7 ± 4.5	0.260	
Total cholesterol (mg/dL)	159.3 ± 24.8	171.3 ± 31.6	185.3 ± 15.9	**0.042**	0.073
Triglycerides (mg/dL)	67.2 (54.5-83)	69.1 (52.9-90.3)	70.2 (46.3-106.4)	0.851	0.998
HDL (mg/dL)	56.5 ± 13.3	52.4 ± 10.4	53.3 ± 13.9	0.279	0.326
LDL (mg/dL)	87.4 ± 21.7	101.4 ± 27.2	117.8 ± 14.2	**0.007**	**0.014**
Glycohemoglobin (%)	5.3 (5.2-5.4)	5.3 (5.2-5.4)	5.3 (5-5.5)	0.919	0.765
Fasting glucose (mmol/l)	4.9 (4.8-5)	5.1 (4.9-5.2)	4.9 (4.6-5.2)	0.147	0.248

rs478621

	CC	CG	GG	P*	P_adj †
N	63	14	0		
Age (yr)	31.8 ± 10.7	34.6 ± 11.7		0.374	
BMI (kg/m²)	26.9 ± 7.2	26.4 ± 5.3		0.771	
Total cholesterol (mg/dL)	165.8 ± 31	172.6 ± 14.2		0.480	0.555
Triglycerides (mg/dL)	70.4 (58.6-84.7)	60.3 (44.8-81.1)		0.440	0.550
HDL (mg/dL)	54.4 ± 12.4	53.5 ± 10.4		0.836	0.574
LDL (mg/dL)	94.3 ± 26.7	106 ± 17.7		0.173	0.178
Glycohemoglobin (%)	5.3 (5.2-5.4)	5.3 (5.1-5.4)		0.883	0.757
Fasting glucose (mmol/l)	4.9 (4.9-5)	5 (4.7-5.3)		0.678	0.763
2-h glucose (mmol/l)	6.5 (6.3-6.7)	6.5 (6.2-6.8)		0.904	0.920
Fasting insulin (pmol/l)	29.7 (24.6-35.9)	32.2 (22.5-46)		0.712	0.263
2-h insulin (pmol/l)	195.2 (169.5-224.9)	207.9 (154.2-280.4)		0.699	0.343
HOMA-IR	1.1 (0.9-1.3)	1.2 (0.8-1.7)		0.693	0.271

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	TT	TC	CC	P*	P_adj †
N	40	28	9		
Age (yr)	32.6 ± 10.9	31.4 ± 10.8	33.8 ± 12.4	0.978	
BMI (kg/m²)	27.1 ± 6.6	27.4 ± 7.9	24 ± 3.3	0.399	
Total cholesterol (mg/dL)	170.6 ± 31.3	164.6 ± 25	159.3 ± 28.6	0.291	0.315
Triglycerides (mg/dL)	79.9 (63.6-100.2)	57.8 (44.5-75.1)	58.2 (36.9-91.9)	0.066	0.119
HDL (mg/dL)	55.1 ± 12.7	52.9 ± 12.2	54.7 ± 8.4	0.732	0.436
LDL (mg/dL)	96.4 ± 25.6	98.2 ± 28.1	91.9 ± 19.6	0.835	0.955
Glycohemoglobin (%)	5.3 (5.2-5.4)	5.3 (5.2-5.4)	5.3 (5.1-5.5)	0.976	0.768
Fasting glucose (mmol/l)	4.9 (4.8-5)	5 (4.8-5.2)	5.1 (4.8-5.4)	0.112	0.059

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	CC	CG	GG	P^*	$P_{adj}^{\dagger}$		AA	AG	GG	P^*	$P_{adj}^{\dagger}$
2-h glucose (mmol/l)	6.4 (6.1-6.6)	6.8 (6.5-7)	6.5 (5.9-7.2)	0.150	0.162	2-h glucose (mmol/l)	6.4 (6.2-6.6)	6.7 (6.3-7)	6.6 (6.2-7)	0.279	0.224
Fasting insulin(pmol/l)	27.8 (22.7-34.1)	37.3 (27-51.7)	21.4 (15.3-29.9)	0.906	0.454	Fasting insulin(pmol/l)	26.3 (21.1-32.7)	34.1 (25.6-45.5)	34.1 (21.3-54.5)	0.152	0.335
2-h insulin (pmol/l)	188.1 (159.9-221.2)	232 (185.8-289.7)	143.6 (93.3-220.9)	0.853	0.874	2-h insulin (pmol/l)	181.3 (154.8-212.3)	213.8 (172.4-265.1)	211.3 (116.4-383.7)	0.252	0.366
HOMA-IR	1 (0.8-1.2)	1.4 (1-1.9)	0.8 (0.6-1.2)	0.791	0.359	HOMA-IR	0.9 (0.8-1.2)	1.3 (0.9-1.7)	1.2 (0.8-2)	0.129	0.295

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

* Not adjusted for age and BMI.

† Adjusted for age and BMI.