CLINICAL STUDY

Evidence for association between paraoxonase 1 gene polymorphisms and polycystic ovarian syndrome in south-west Chinese women

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Abstract

Objective: The aim of this study was to investigate the relationship between 192Q/R and 55L/M polymorphisms of paraoxonase 1 (PON1) gene and polycystic ovarian syndrome (PCOS) in Chinese women.

Design: A case–control study.

Methods: A total of 1113 subjects (610 patients with PCOS and 503 control women) from a population of Chinese Han nationality in Chengdu area were included in this study. PON1 genotypes were studied using PCR and restriction fragment length polymorphism analysis. Clinical and metabolic parameters were analyzed.

Results: The frequencies of PON1 192RR genotype and R allele were significantly higher in patients with PCOS than in control women (44.6 vs 36.4%, 0.667 vs 0.610 respectively). The 192RR genotype remained a significant predictor for PCOS (odds ratio $_{RR/QR+QQ}$: 1.656, 95% confidence interval: 1.156–2.371) in prognostic models including age, body mass index, insulin resistance index, triglyceride, HDL, and LDL as covariates. Compared with patients with QQ genotype, patients with RR or QR genotype had significantly higher waist circumference and fasting insulin and triglyceride levels, patients with RR genotype had significantly higher waist-to-hip ratio, and patients with QR genotype had significantly higher homeostasis model assessment of insulin resistance. Such relationships were not detected in the control women. No significant differences were found in the frequencies of PON1 55L/M genotype and allele between PCOS and control groups.

Conclusions: The 192Q/R, but not 55L/M, polymorphism in PON1 gene is associated with the risk of PCOS in south-west Chinese women.

European Journal of Endocrinology 166 877–885

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous female endocrine metabolic disorder affecting 5–10% of women of reproductive age and may account for anovulatory infertility and threaten long-term health (1, 2, 3). PCOS represents a broad spectrum of metabolic alterations such as glucose intolerance, insulin resistance, dyslipidemia, obesity (especially the visceral phenotype), and increased oxidative stress (2, 4, 5, 6). Consequently, there is an increase in the risk factors for cardiovascular disease (1, 7), including hyperandrogenism (HA), impaired fibrinolysis, increased systemic low-grade inflammation, an elevated prevalence of subclinical and clinical atherosclerosis (1, 6), an increased prevalence of impaired glucose tolerance, and type 2 diabetes mellitus (4, 8). The etiology of PCOS is not clear, but studies have suggested that PCOS appears to have a complex, multifactorial etiology in

which a variety of predisposing genes interact with environmental factors to produce the disease (9, 10).

Serum paraoxonase 1 (PON1) is a calcium-dependent esterase/lactonase, which is and mainly synthesized by the liver and circulates within HDL particles (11, 12). PON1 has PON, arylesterase, and lactonase activities (11). The enzyme not only hydrolyzes several organophosphorus insecticides and nerve agents, which is involved in the protection against xenobiotic toxicity (12), but also inhibits LDL oxidation, increases macrophage-associated cholesterol efflux, and possesses antioxidant, anti-inflammatory, and anti-atherogenic properties (11, 13). Recent studies showed that the lactonase activity of PON1 could play important roles in hydrolyzing and detoxifying oxidative stress mediators. In addition, PON1 is able to hydrolyze homocysteine thiolactone and thus inhibit homocysteinylation of proteins, which could be involved

in protecting against protein inactivation, cell damage, and atherosclerosis (12).

Several polymorphisms in the exons and promoter region of *PON1* gene have been reported to influence its activity or concentration. There are two common polymorphisms in the coding region, which lead to a glutamine→arginine substitution at position 192 (192Q/R) and leucine→methionine substitution at position 55 (55L/M). The T to C exchange in position –108 is one of the several polymorphisms identified in the promoter region of *PON1*. The 192Q/R (*rs662*) polymorphism has been shown to affect PON1 activities in a substrate-dependent manner such that paraoxon (14, 15, 16) is hydrolyzed *in vitro* more efficiently by the R isoform, whereas diazoxon, soman, sarin (15), and lipid peroxides (11, 12, 17) are hydrolyzed more rapidly by the Q isoform, but there are contradictory data about the relationship between 192 isoforms and the rates of phenylacetate hydrolysis (14, 15, 18). Both the *PON1* 55L/M (*rs854560*) and the *PON1* –108C/T (*rs705379*) variants are associated with variations in concentrations (11, 12, 18). As genetic factors, including polymorphism, were found to explain more than 60% phenotypic variance in PON1 activity (14), *PON1* genetic factors may have an important effect on the physiological function of PON1 and its association with diseases. Studies showed that some of the *PON1* polymorphisms were associated with several disorders: the *PON1* 192Q/R polymorphism was associated with coronary heart disease (19, 20), ischemic stroke (21), Alzheimer's disease (11), and chronic kidney disease (22); the *PON1* 55L/M polymorphism was associated with Parkinson's disease (23); and the *PON1* –108C/T polymorphism was associated with increased serum glucose concentrations in nondiabetic patients (24).

Recent evidence has shown that extensive oxidative stress and the state of low-grade inflammation may play important roles in HA, insulin resistance, and metabolic and cardiovascular long-term complications of PCOS (6, 25). HDLs possess antioxidant, anti-inflammatory, and anti-atherogenic properties, while the enzymes associated with HDL including PON1 play a crucial role in antioxidant functions of HDL (13). However, up to now, only a few reports regarding the possible connection between the *PON1* and PCOS are available. Dursun *et al.* (26) reported that serum PON activity was decreased in patients with PCOS compared with control women, and San Millan *et al.* (27) reported that homozygosity for T alleles of the –108C/T, but not 192Q/R and 55M/L, polymorphism in *PON1* was more frequent in women with PCOS (*n*=72) compared with nonhyperandrogenic control women (*n*=42) in Spain. In contrast with the previous reports, our recent study showed that the –108C/T polymorphism in the *PON1* gene was not associated with the risk of PCOS in Chinese women (28). Chen *et al.* (29) studied a relationship between 23 single nucleotide polymorphisms in the *PON1* gene and PCOS in north Chinese

women in a genome-wide association study, but the study did not include *rs662* and *rs854560* sites. As genotype and allele frequencies for *PON1* gene polymorphisms differ greatly among ethnic groups (12, 30), it is important to address whether there are any relationships between the 192Q/R and the 55L/M polymorphisms and PCOS in Chinese women. Therefore, in this study, we investigated the genotype distribution of *PON1* 192Q/R and 55L/M polymorphisms in women from healthy and PCOS populations in a well-characterized Chinese population using relatively large sample sizes (610 cases and 503 controls).

Materials and methods

Subjects

Six hundred and ten patients with PCOS and 503 control women between 17 and 45 years of age during 2006 to 2011 were studied in south-west China. For genetic association study, we included all the patients (*n*=610) and controls (*n*=503). For association study between *PON1* genotypes and hormonal, biochemical, and metabolic parameters, all recruited women were not taking any medication known to affect carbohydrate or lipid or hormone metabolism for at least 3 months before the study and were studied during the follicular phase of their menstrual cycle (progesterone level <3 ng/ml). In amenorrheic women, recent ovulation was excluded by progesterone measurement (<3 ng/ml). Therefore, the subgroups of patients and controls for the biochemical and metabolic measurements consisted of 406 and 341 subjects respectively. In addition, as 43 patients with PCOS and 112 control women did not do oral glucose tolerance test tests in the metabolic analysis groups, the subgroups of the patients and the controls for 2 h insulin and glucose measurements consisted of 363 and 229 subjects respectively. In general, the subgroups of the patients and the controls were comparable to the respective groups of total patients and controls in terms of clinical characteristics such as age, body mass index (BMI), Ferriman–Gallwey score (F-G score), systolic and diastolic blood pressure (SBP and DBP), and the genotype frequencies of *PON1* 192Q/R and 55L/M. All study participants gave their informed consent, and the study was approved by our institutional review board.

All patients with PCOS were recruited from the Outpatient Department of Reproductive Endocrinology of West China Second University Hospital at Sichuan University in Chengdu. Each patient with PCOS met the diagnostic criteria for PCOS based on the revised 2003 Rotterdam ESHRE/ASRM consensus criteria and exclusion of related disorders (3). Oligo- and/or anovulation was assessed as oligomenorrhea, i.e. less than eight cycles per year. Sonographic diagnosis of PCOS was confirmed if there were 12 or more follicles in each

ovary measuring 2–9 mm in diameter and/or increased ovarian volume (>10 ml). Hyperandrogenemia was assessed as total testosterone (TT) levels above the 95th percentile of the levels (0.75 ng/ml) detected in a group of normal menstruating women with normal cycles and/or clinically by the presence of obvious acne and/or hirsutism with the F-G score of more than 6 (31, 32). The diagnosis of PCOS was based on a patient having two of these three findings, with exclusion of congenital adrenal hyperplasias, androgen-secreting tumors, and Cushing's syndrome.

The control group consisted of infertile women due to salpingian obstruction or the husband's infertility and recruited from the above-mentioned Outpatient Department of the hospital during the same period. All women were clinically healthy who had regular menstrual cycles (shorter than 35 days), exhibited normal circulating androgen levels (TT <0.75 ng/ml), and the absence of obvious acne or hirsutism (F-G score <6) on physical examination.

None of the subjects had clinically evident chronic or acute diseases such as infection, tumors, thyroid dysfunction, cardiovascular disease, endometriosis, hyperprolactinemia, hypogonadotropic hypogonadism, or premature ovarian failure.

Clinical and anthropometrical variables including waist circumference, hip circumference, waist-to-hip ratio (WHR), BMI (kg/m²), blood pressure, and the degree of hirsutism and acne were measured or assessed in all subjects.

Blood samples were obtained in the morning after overnight fasting. Blood samples for genotype analysis were collected in tubes containing EDTA, centrifuged at 1500 g for 15 min, and blood cells were stored at 4 °C.

Genotype analysis

Genomic DNA was isolated from the peripheral blood leukocytes of subjects according to the method of Erlich (33). The *PON1* 192Q/R and 55L/M genotypes were determined by PCR amplification and restriction analysis. PCR (total volume 25 µl) contained genomic DNA template (2.0 µl, about 30–80 ng), 200 µM each of dNTP, 1.5 mM MgCl₂, 0.75 U recombinant Taq polymerase (MBI Fermentas, Vilnius, Lithuania), and 0.30 µM of each primer. For 192Q/R genotype, a 99 bp fragment was amplified using primers (34): forward, 5'-TAT TGT TGC TGT GGG ACC TGA G-3' and reverse 5'-CAC GCT AAA CCC AAA TAC ATC TC-3'; for 55L/M genotype, a 172 bp fragment was amplified using primers: forward, 5'-CCT GCA ATA ATA TGA AAC AAC CTG-3' and reverse 5'-TGA AAG ACT TAA ACT GCC AGT C-3' (Genecor Biotech Co. Ltd., Shanghai, China). The PCR was performed in a MyCycler thermal cycler (Bio-Rad Laboratories, Inc.) as follows: pre-denaturation at 95 °C for 3 min, followed by 32 cycles of 45 s at 95 °C, 45 s at 61 °C, and 1 min at 72 °C for 192Q/R genotyping; 30 cycles of 45 s at 95 °C, 45 s at

59 °C, and 1 min at 72 °C for 55L/M genotyping; ending with a single 7 min extension step at 72 °C. Four to five microliters of 99 or 172 bp PCR products were digested with 1U BspPI (AlwI) and Hin1II (NlaIII) (MBI Fermentas) for 4–16 h at 37 °C (55 °C for BspPI) respectively, and digestion resulted in 64 and 35 bp fragment for 192R allele and in a nondigested 99 bp fragment for 192Q allele; 106 and 66 bp fragment for 55M allele and in a nondigested 172 bp fragment for 55L allele. The products were analyzed by electrophoresis on a 10% PAGE gel or 3.0% agarose gel and visualized by staining with ethidium bromide. For the purpose of quality control of the genotyping, 30% of DNA samples were genotyped again by the different operator. Eleven DNA samples for 192Q/R genotype and nine DNA samples for 55L/M genotype with low PCR yield were re-amplified.

Analysis of hormonal and metabolic profiles

The levels of serum FSH, LH, TT, estradiol (E₂), progesterone (P), prolactin, cortisol, TSH, and the concentrations of fasting plasma insulin were measured by chemiluminescence assays (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA). The concentrations of plasma total cholesterol, HDL-C, LDL-C, and triglyceride were measured by enzymatic assay (Boehringer) using a Hitachi 7600-010 automatic analyzer. Fasting glucose was measured by the glucose oxidase technique (Roche Diagnostics GmbH). The intra- and interassay coefficients of variation for all measurements were <5 and 10% respectively.

The estimate of insulin resistance was calculated by the homeostasis model assessment of insulin resistance index (HOMA-IR = fasting glucose (mmol/l) × fasting insulin (µU/ml)/22.5) (35).

Atherogenic index (AI) was calculated using the following equation: AI = (TC – (HDL-C))/(HDL-C) (36).

Statistical analysis

Data were presented as mean ± s.d. Differences in variables were evaluated by the independent sample *t*-test between PCOS and control subjects. Variables with asymmetric distribution were evaluated by nonparametric tests (Mann–Whitney *U* test). χ^2 analysis was used to test deviations of genotype distribution from Hardy–Weinberg equilibrium and to determine allele or genotype frequencies between patients and controls. Odds ratio (OR) and 95% confidence intervals (CI) were calculated to test relative risk for PCOS associated with R allele by χ^2 analysis and logistic regression methods. ANOVA was used to estimate the effect of *PON1* genotypes on clinical and metabolic parameters. A value of *P* < 0.05 was considered to be statistically significant. All statistical analyses were performed using Statistical Program for Social Sciences (SPSS) 13.0 for Windows (Chicago, IL, USA).

Results

Clinical and biochemical characteristics of the study population

In accordance with the revised 2003 Rotterdam ESHRE/ASRM consensus criteria, there were 251 cases (41.15%) with oligo- and/or anovulation (OA) + HA + polycystic ovaries (PCO), 190 cases (31.15%) with OA + PCO, 134 cases (22%) with OA + HA, and 35 cases (5.7%) with HA + PCO in the PCOS group.

As shown in Table 1, BMI, waist circumference, WHR, F-G score, SBP, DBP, average ovarian volume, and the day of menstrual cycle at sampling were significantly higher and age was significantly lower in the PCOS group compared with the control group.

P, TT, and LH levels; the ratio of LH to FSH; fasting and 2 h insulin and glucose concentration; HOMA index; TC, TG, and LDL-C levels; AI; and the ratio of TG to HDL-C were significantly higher, and FSH and HDL-C levels were significantly lower in patients with PCOS in comparison with the control group, although there was no significant difference with respect to E₂ levels between the two groups (Table 2).

Distribution of PON1 192Q/R and 55L/M genotypes and alleles

Genotypic and allelic frequencies of PON1 192Q/R and 55L/M gene sites are summarized in Table 3. Genotypic distributions were in Hardy–Weinberg equilibrium in the PCOS and control groups. No significant differences were found in the frequencies of PON1 55L/M genotypes and alleles between PCOS and control groups ($P > 0.05$). However, the prevalence of the PON1 192RR genotype was significantly more frequent in patients with PCOS compared with those in the control women (44.6 vs 36.4%, $P = 0.016$). There was also a significant difference in PON1 192Q/R allelic frequencies between PCOS and control subjects (0.667 vs 0.610 for R allele, $P = 0.005$). Logistic regression

analysis adjusted for age, BMI, LDL, HDL, TG, and HOMA-IR also showed that the relative risk of subjects with RR genotypes for PCOS was higher compared with those with RR + QR genotype (OR = 1.656, 95% CI: 1.156–2.371, $P = 0.006$).

We also analyzed the frequencies of haplotype combination genotypes of PON1 55M/L and 192Q/R in patients with PCOS and the control women. The results show that the frequencies of haplotype combination L-R/L-R genotype is 44.8% in patients with PCOS and significantly higher than 36.4% in the control women, and the haplotype containing both variations M-R is not observed (Table 4). The multinomial logistic regression analysis shows that the haplotype combination L-R/L-R genotype is a risk factor for PCOS (OR = 1.646, 95% CI: 1.102–2.460, $P = 0.015$, Table 4) when the L-Q/L-Q wild-type is the reference category.

Effects of PON1 192Q/R and 55L/M genetic variants on clinical, hormonal, and metabolic parameters

Table 5 shows the influences of PON1 192Q/R genetic variant on clinical, hormonal, and metabolic parameters. Compared with patients with QQ genotype, patients with RR or QR genotype had significantly higher waist circumference, fasting insulin, and TG levels ($P < 0.05$) and tended to have higher BMI, AI, and the ratio of TG to HDL-C ($P < 0.10$); patients with RR genotype had significantly higher WHR ($P < 0.05$); and patients with QR genotype had significantly higher HOMA-IR ($P < 0.05$). However, there were no significant differences in clinical, biochemical, and metabolic parameters according to PON1 192Q/R genotypes in the control women ($P > 0.05$). In addition, in subjects with normal body weight (BMI < 25), compared with the patients with QQ genotype ($n = 37$), RR genotype ($n = 134$), or QR genotype ($n = 124$) have significantly higher TT levels (0.72 ± 0.23 or 0.70 ± 0.20 vs 0.61 ± 0.22 ng/ml, all $P < 0.05$), and the patients with RR genotype have higher WC (75.45 ± 7.22 vs 72.70 ± 6.27 cm, $P < 0.05$).

Patients with 55LM heterozygotes had lower F-G score ($P = 0.011$) and tended to have lower BMI and 2 h insulin levels when compared with patients with 55LL homozygotes ($P < 0.07$). No significant differences in clinical, biochemical, and metabolic parameters according to PON1 55M/L genotypes were detected in the control women ($P > 0.05$) (Supplementary Table 1, see section on supplementary data given at the end of this article).

We further analyzed the relationships between the haplotype combination genotypes of PON1 55M/L and 192Q/R and clinical and biochemical parameters in patients with PCOS. The results show that the patients with L-R/L-R genotype ($n = 186$) have significantly higher WC and WHR when compared with the L-Q/L-Q

Table 1 Clinical characteristics in patients with PCOS and control women. Values are presented as mean $\pm$ s.d.

	Controls ($n = 503$)	PCOS ($n = 610$)	<i>P</i>
Age (years)	28.25 ± 4.12	24.72 ± 4.02	0.000
BMI (kg/m ²)	20.92 ± 2.67	22.63 ± 4.01	0.000
WC (cm)	73.61 ± 7.71	78.21 ± 10.95	0.000
WHR	0.82 ± 0.06	0.85 ± 0.07	0.000
F-G score	0.20 ± 0.65	1.68 ± 2.03	0.000
SBP (mmHg)	113.12 ± 10.69	114.51 ± 10.42	0.032
DBP (mmHg)	74.25 ± 8.05	75.51 ± 7.90	0.009
Ovarian volume (ml)	7.32 ± 2.54	9.81 ± 4.34	0.000
Menstrual cycle at sampling (days)	12.51 ± 14.09	52.18 ± 59.54	0.000

WC, waist circumference; WHR, waist-to-hip ratio.

Table 2 Hormonal levels and metabolic profile in patients with PCOS and control women. Values are presented as mean $\pm$ s.d.

	Controls (n=341)	PCOS (n=406)	P
Age (years)	27.92 $\pm$ 4.06	24.71 $\pm$ 4.03	0.000
BMI (kg/m ²)	20.79 $\pm$ 2.60	22.88 $\pm$ 4.22	0.000
Menstrual cycle at sampling (days)	6.94 $\pm$ 4.24	62.75 $\pm$ 66.24	0.000
Hormonal levels			
E ₂ (pg/ml)	95.42 $\pm$ 104.78	85.41 $\pm$ 84.40	0.964
P (ng/ml)	0.77 $\pm$ 0.39	0.91 $\pm$ 0.63	0.003
TT (ng/ml)	0.43 $\pm$ 0.15	0.70 $\pm$ 0.22	0.000
LH (mIU/ml)	7.72 $\pm$ 9.30	14.02 $\pm$ 11.89	0.000
FSH (mIU/ml)	6.27 $\pm$ 2.66	5.76 $\pm$ 2.42	0.011
LH/FSH	1.27 $\pm$ 1.25	2.48 $\pm$ 1.33	0.000
Metabolic profile			
Fasting Ins (μ U/ml)	9.27 $\pm$ 5.26	14.65 $\pm$ 10.10	0.000
Fasting Glu (mmol/l)	5.31 $\pm$ 0.49	5.41 $\pm$ 0.61	0.015
2 h Ins (μ U/ml) ^a	51.27 $\pm$ 42.00	102.44 $\pm$ 81.93	0.000
2 h Glu (mmol/l) ^a	6.04 $\pm$ 1.32	7.12 $\pm$ 2.31	0.000
HOMA-IR	2.23 $\pm$ 1.42	3.65 $\pm$ 3.08	0.000
TG (mmol/l)	1.14 $\pm$ 1.01	1.36 $\pm$ 1.09	0.000
TC (mmol/l)	4.23 $\pm$ 0.72	4.40 $\pm$ 0.84	0.002
HDL-C (mmol/l)	1.51 $\pm$ 0.32	1.39 $\pm$ 0.36	0.000
LDL-C (mmol/l)	2.25 $\pm$ 0.63	2.55 $\pm$ 0.77	0.000
AI	1.89 $\pm$ 0.70	2.34 $\pm$ 0.99	0.000
TG/HDL-C	0.75 $\pm$ 0.54	1.15 $\pm$ 1.40	0.001

AI, atherogenic index. Ins, insulin; Glu, glucose.

^aControl (n=229), PCOS (n=363).

wild types (n=38) or the M-Q/L-Q genotypes (n=4) (WC: 80.26 $\pm$ 11.14 vs 75.11 $\pm$ 8.54 or 68.75 $\pm$ 5.56 cm; WHR: 0.86 $\pm$ 0.07 vs 0.83 $\pm$ 0.06 or 0.77 $\pm$ 0.04; all $P < 0.05$). The patients with L-R/L-R genotype or M-Q/L-R genotype (n=19) have higher TT levels when compared with the M-Q/L-Q genotypes (0.71 $\pm$ 0.22 or 0.72 $\pm$ 0.23 vs 0.48 $\pm$ 0.26 ng/ml, all $P < 0.05$) but not significant differences in glucose and lipid metabolic parameters ($P > 0.05$).

Discussion

In this study, we show that an association exists between the *PON1* 192Q/R, but not 55L/M, genetic polymorphism and the risk of PCOS in Chinese women, suggesting that this polymorphism may predispose to the disease susceptibility and potentially link to related complications responsible for oxidative stress and inflammation such as an increased risk for type 2 diabetes mellitus and/or future cardiovascular diseases in the patients.

Although the potential contributions of the genetic components to PCOS remain to be characterized, the increased systemic low-grade inflammation, one of the important pathological conditions of the disease, is thought to be multifactorial, involving the interaction of genetic and environmental influences. Human and animal studies strongly support the hypothesis that oxidative modification of lipoproteins, especially LDL,

plays an important role in the onset of chronic inflammation and the pathogenesis of atherosclerosis (13). Therefore, mechanisms preventing oxidation of lipoproteins appear to be anti-inflammatory and anti-atherogenic. HDLs were believed to be one of the most important defenses against lipid peroxides in the circulatory system because the enzymes associated with HDL, including *PON1* and platelet-activating factor acetylhydrolase (PAF-AH), play a crucial role in antioxidant functions of HDL (13). Our previous studies showed that HDL-associated PAF-AH activity was decreased in women with PCOS and the *PAF-AH* G994 $\rightarrow$ T gene mutation, which leads to completely abolish PAF-AH activity, and is a risk factor for PCOS (32, 37, 38). In this study, we found that the 192Q/R polymorphism in *PON1* gene was associated with the risk of PCOS in Chinese women, suggesting that the dysfunction of *PON1* may also be involved in the pathophysiological process of the disease.

There is sufficient evidence that *PON1* status on HDL in circulation is regulated by *PON1* genetic polymorphisms (12). Studies have found that the *PON1* 192Q/R polymorphism itself does not affect *PON1* protein concentrations but greatly influences *PON1* activities, especially PON and lactonase activities (12, 39). HDL containing R192 *PON1* isoform presents the highest PON activity, which is able to hydrolyze organophosphorus insecticide paraoxon, but the lowest lactonase activity, which is associated with hydrolysis of lipid peroxides (14). This suggests that the effects of Q192R polymorphism on PON and lactonase activities of *PON1* are opposite. In addition, there are contradictory data about the rates of *PON1* phenylacetate hydrolysis (arylesterase activity), which is in a linear relationship with *PON1* expression level (18, 40). Phenylacetate is hydrolyzed at

Table 3 Frequencies of *PON1* genotype and allele in patients with PCOS compared with control women. Data of genotype are presented as number (%) of patients or controls.

	Controls (n=503)	PCOS (n=610)	χ^2	<i>P</i>
Genotype				
192				
<i>QQ</i>	72 (14.3%)	68 (11.1%)	8.247	0.016
<i>QR</i>	248 (49.3%)	270 (44.3%)		
<i>RR</i> ^a	183 (36.4%)	272 (44.6%)		
55				
<i>LL</i>	472 (93.8%)	569 (93.3%)	0.142	0.706
<i>LM</i>	31 (6.2%)	41 (6.7%)		
<i>MM</i>	0 (0.0%)	0 (0.0%)		
Allele frequency				
192				
<i>Q</i>	0.390	0.333	7.755	0.005
<i>R</i>	0.610	0.667		
55				
<i>L</i>	0.969	0.966	0.137	0.711
<i>M</i>	0.031	0.034		

^aOdds ratio (OR)_{RR/QR+QQ} 1.226, 95% confidence interval 1.060–1.418, $\chi^2 = 7.686$, $P = 0.006$.

Table 4 Frequencies of haplotype combination genotypes of *PON1* 55M/L and 192Q/R in patients with PCOS and the control women. Odds ratio (OR) and 95% confidence interval (CI) were calculated in a multinomial logistic regression model wild-type L-Q/L-Q as the reference category. A haplotype containing both variations 55M–192R was not observed.

Haplotype combination	Controls (n=503)	PCOS (n=610)	OR	95% CI	P
L-Q/L-Q	64 (12.7%)	58 (9.5%)	1.00	–	–
L-R/L-R	183 (36.4%)	273 (44.8%)	1.646	1.102–2.460	0.015
L-R/L-Q	225 (44.7%)	239 (39.2%)	1.172	0.786–1.747	0.436
M-Q/L-R	23 (4.6%)	31 (5.1%)	1.487	0.780–2.837	0.228
M-Q/L-Q	8 (1.6%)	9 (1.5%)	1.241	0.449–3.431	0.667

L-Q, the haplotype containing both variations 55L–192Q; L-R, the haplotype containing both variations 55L–192R; M-Q, the haplotype containing both variations 55M–192Q. Data of haplotype combinations are presented as number (%) of patients or controls, χ^2 test: $\chi^2=9.526$, $P=0.049$.

approximately the same rate by both Q and R isoforms (15), more rapidly by the Q isoform (18), or more rapidly by the R isoform (14). As *PON1* R192 allele has been reported to be in linkage disequilibrium with the *PON1* –108C allele (14, 18), it is possible that the *PON1* –108C allele that increases *PON1* expression may partly compensate for the *PON1* arylesterase activities afforded by the R isoform in some cases.

Recent studies have shown that the native activity of *PON1* is lactonase activity and the physiological function of *PON1* *in vivo* is the degradation of oxidized lipids (11). HDL with *PON1* R192 isoform has lower lactonase activity and is less efficient at retarding the

oxidation of LDL when compared with HDL with Q192 *PON1* isoform (12, 41). These results indicate that 192RR homozygosity has the lowest activity for hydrolyzing lipid peroxides, thus lowest anti-inflammatory and anti-atherogenic property. It has been reported that increased oxidative stress is able to induce insulin resistance by mediating serine phosphorylation of the insulin receptor substrates (IRS)-1 and -2, which leads to an abrogation of insulin signaling via its receptor (25). Our results that patients with 192R alleles had higher (or tended to increase) fasting insulin levels, HOMR-IR, and the ratio of TG to HDL-C, a marker of the small dense LDL particles that predispose to

Table 5 Clinical characteristics, hormonal levels, and metabolic profile according to *PON1* Q192R genotype in patients with PCOS and control women. Values are presented as mean $\pm$ s.d.

	Control group			PCOS group		
	QQ (n=54)	QR (n=173)	RR (n=114)	QQ (n=43)	QR (n=178)	RR (n=185)
Clinical characteristics						
Age (years)	27.26 $\pm$ 4.42	27.95 $\pm$ 4.13	28.19 $\pm$ 3.77	24.02 $\pm$ 4.14	25.07 $\pm$ 4.02	24.51 $\pm$ 4.00
BMI (kg/m ²)	21.06 $\pm$ 2.81	20.62 $\pm$ 2.45	20.92 $\pm$ 2.72	21.68 $\pm$ 3.75	22.99 $\pm$ 4.20	23.05 $\pm$ 4.32
WC (cm)	74.43 $\pm$ 8.20	73.27 $\pm$ 7.48	72.77 $\pm$ 7.48	74.58 $\pm$ 8.38	78.68 $\pm$ 11.92 ^a	80.36 $\pm$ 11.12 ^a
WHR	0.82 $\pm$ 0.06	0.82 $\pm$ 0.06	0.82 $\pm$ 0.07	0.83 $\pm$ 0.06	0.85 $\pm$ 0.08	0.86 $\pm$ 0.07 ^a
F-G score	0.11 $\pm$ 0.42	0.26 $\pm$ 0.71	0.27 $\pm$ 0.81	1.31 $\pm$ 1.60	1.78 $\pm$ 2.16	1.63 $\pm$ 1.97
Ovarian volume (ml)	8.15 $\pm$ 3.57	7.27 $\pm$ 2.29	7.95 $\pm$ 2.54	9.47 $\pm$ 4.30	9.74 $\pm$ 4.23	10.46 $\pm$ 4.83
Hormonal levels						
TT (ng/ml)	0.43 $\pm$ 0.16	0.42 $\pm$ 0.14	0.45 $\pm$ 0.16	0.66 $\pm$ 0.27	0.70 $\pm$ 0.20	0.71 $\pm$ 0.22
FSH (mIU/ml)	6.03 $\pm$ 1.84	6.43 $\pm$ 3.10	6.14 $\pm$ 2.19	6.08 $\pm$ 1.72	5.83 $\pm$ 2.89	5.61 $\pm$ 2.03
LH (mIU/ml)	6.32 $\pm$ 3.19	8.29 $\pm$ 11.01	7.48 $\pm$ 8.12	14.42 $\pm$ 7.64	13.88 $\pm$ 10.05	14.06 $\pm$ 14.17
LH/FSH	1.12 $\pm$ 0.66	1.28 $\pm$ 1.15	1.27 $\pm$ 1.26	2.44 $\pm$ 1.36	2.51 $\pm$ 1.23	2.47 $\pm$ 1.43
Metabolic profile						
Ins0 (μ U/ml)	8.89 $\pm$ 3.92	9.23 $\pm$ 5.40	9.36 $\pm$ 5.61	11.24 $\pm$ 6.69	15.29 $\pm$ 11.69 ^a	14.79 $\pm$ 9.80 ^a
Ins120 (μ U/ml) ^b	43.13 $\pm$ 23.89	51.47 $\pm$ 36.39	55.10 $\pm$ 54.89	94.52 $\pm$ 72.71	99.74 $\pm$ 74.99	106.88 $\pm$ 90.06
Glu0 (mmol/l)	5.25 $\pm$ 0.46	5.35 $\pm$ 0.44	5.26 $\pm$ 0.56	5.40 $\pm$ 0.97	5.44 $\pm$ 0.62	5.37 $\pm$ 0.47
Glu120 (mmol/l) ^b	5.79 $\pm$ 1.34	6.13 $\pm$ 1.35	6.03 $\pm$ 1.26	7.16 $\pm$ 3.42	7.03 $\pm$ 2.02	7.19 $\pm$ 2.26
HOMA-IR	2.10 $\pm$ 1.00	2.26 $\pm$ 1.45	2.25 $\pm$ 1.55	2.85 $\pm$ 2.53	3.88 $\pm$ 3.61 ^a	3.62 $\pm$ 2.60
TG (mmol/l)	0.93 $\pm$ 0.39	1.07 $\pm$ 1.30	1.04 $\pm$ 0.66	1.11 $\pm$ 0.47	1.41 $\pm$ 1.24 ^a	1.36 $\pm$ 1.02 ^a
TC (mmol/l)	4.10 $\pm$ 0.70	4.29 $\pm$ 0.74	4.21 $\pm$ 0.68	4.33 $\pm$ 0.72	4.47 $\pm$ 0.90	4.36 $\pm$ 0.81
HDL-C (mmol/l)	1.50 $\pm$ 0.31	1.51 $\pm$ 0.32	1.53 $\pm$ 0.32	1.48 $\pm$ 0.43	1.38 $\pm$ 0.32	1.38 $\pm$ 0.38
LDL-C (mmol/l)	2.25 $\pm$ 0.55	2.42 $\pm$ 0.67	2.30 $\pm$ 0.59	2.44 $\pm$ 0.66	2.60 $\pm$ 0.82	2.53 $\pm$ 0.75
AI	1.79 $\pm$ 0.48	1.95 $\pm$ 0.78	1.85 $\pm$ 0.65	2.10 $\pm$ 0.79	2.39 $\pm$ 0.97	2.36 $\pm$ 1.04
TG/HDL-C	0.66 $\pm$ 0.38	0.86 $\pm$ 2.08	0.75 $\pm$ 0.67	0.87 $\pm$ 0.59	1.18 $\pm$ 1.44	1.14 $\pm$ 1.40

^a $P < 0.05$ compared with QQ genotype subgroup in patients with PCOS.

^bControl: QQ (n=38), QR (n=115), RR (n=76); PCOS: QQ (n=40), QR (n=154), RR (n=169). Ins, insulin; Glu, glucose.

increase susceptibility to oxidative stress compared with patients with 192QQ genotype, suggest that the patients with 192R alleles may have relatively higher oxidative stress levels, which may induce insulin resistance. San Millan *et al.* (27) reported that homozygosity for T alleles (decreased PON1 gene expression) of the PON1 –108C/T polymorphism presented with increased hirsutism scores and serum testosterone concentrations when compared with carriers of –108C alleles. Our findings that in patients with normal body weight (BMI <25), the carriers with 192R alleles have significantly higher TT levels when compared with the patients with QQ genotype, prompt that 192R allele may also contribute to HA in these patients. In addition, studies have shown that 192R alleles were associated with increased risk of cardiovascular diseases (20). In this study, compared with patients with 192QQ genotype, the patients with 192R alleles present with greater abdominal obesity and higher TG levels and tended to increased BMI and AI, suggesting that 192R allele may also be associated with increased risks of metabolic and cardiovascular long-term complications of PCOS.

The PON1 55L/M influences both PON1 concentrations and activities. L allele carriers have significantly higher PON1 mRNA and protein levels (12). Compared with 55MM genotype, 55LL homozygotes had higher PON and lactonase activities (14). However, there are evidences to show that the effects of PON1 55L/M polymorphism on PON1 concentrations and activities is not due to the amino acid change but is, rather, largely due to linkage disequilibrium with the 192Q/R variants and the –108 regulatory region polymorphism (14, 18). In this study, the similar frequencies of PON1 55L/M genotypes and alleles were shown between PCOS and control groups, suggesting that PON1 55L/M genetic polymorphism was not associated with the risk of PCOS. However, we found that F-G score was lower, and BMI and 2 h insulin levels tended to be decreased in patients with 55LM genotype when compared with patients with 55LL genotype, implying that M alleles seemed to have a beneficial effect on glucose metabolism and HA associated with phenotypes such as F-G score. Consistent with Cascorbi's findings (34), in this study, we found that a strong linkage disequilibrium exists between the 55M variant and the 192Q variant in PON1 gene because we did not observe the haplotype containing both variations 55M–192R. Moreover, we found that patients with haplotype combination L-Q/L-Q or M-Q/L-Q genotype had lower WC, WHR, and TT levels when compared with the L-R/L-R genotypes, suggesting that the effects of M alleles on F-G score and glucose metabolism might at least partly be due to a linkage disequilibrium between the 55M allele and the 192Q allele in patients with PCOS. It is possible that the 192Q allele that increases PON1 lactonase activity may partly compensate for the low protein expression levels of 55M alleles. It should be noted that there is

a relatively low frequency of PON1 55M variant in Chinese when compared with the frequency in people of European origin (34); further studies in other ethnic groups are needed to evaluate a possible effect of the PON1 55L/M polymorphism for patients with PCOS.

In addition, the allele frequencies for PON1 polymorphisms differ among ethnic groups (12, 18, 30). Environmental factors such as smoking, high-fat diet, and consumption of antioxidants may also affect PON1 status on HDL in circulation or PON1 expression (42). Therefore, it is possible that the relationships between PON1 polymorphism and PON1 activity or disease may be different among different populations.

Limitations of this study were that we could not determine PON1 status and the oxidative stress parameters. Further study to detect PON1 lactonase activity or/and protein levels, and the oxidative stress parameters, such as plasma levels of oxidized LDL, small dense LDL particles, malondialdehyde, and oxidative lipids, in patients with different genotypes may help to provide clues to the mechanisms responsible for the genetic association.

In summary, this study reports that the PON1 192Q/R, but not 55L/M, genetic polymorphism is associated with PCOS among Chinese women. As this polymorphism has been shown to be associated with the increased susceptibility to oxidative stress and inflammation, which may contribute to both the pathogenesis of PCOS and the increased risks for type 2 diabetes mellitus and future cardiovascular diseases, antioxidant therapies and anti-inflammatory, as well as other measures as changes in diet and lifestyle, might be beneficial for women with PCOS and the effective preventive strategies against major complications in the patients.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-11-0986>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by Chinese National Natural Science Foundation (81070463), Program for Changjiang Scholars and Innovative Research Team in University (IRT0935), and Research Seed Fund from West China Second Hospital of Sichuan University (to H Bai).

Author contribution statement

P Fan designed the experiments, analyzed the data, and wrote the paper. H Liu was responsible for patient screening. Y Wang, J Zhang, and F Zhang collected human samples and took part in the experiments. H Bai analyzed the results and revised the paper. All authors discussed the results and commented on the manuscript.

Acknowledgements

We thank patients with PCOS and control women who donated blood samples for this study. We are thankful to You Li, De Hua Wan, Qi Song, and Qin Su for work performed to support this study.

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Received 15 November 2011

Revised version received 15 January 2012

Accepted 2 February 2012