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Authors

Enkhmaa, Byambaa
Anuurad, Erdembileg
Zhang, Wei
et al.

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Lipoprotein(a) and Apolipoprotein(a) in Polycystic Ovary Syndrome

Byambaa Enkhmaa[*], **Erdembileg Anuurad**[*], **Wei Zhang**[*], **Adnan Abbuthalha**[*], **Parneet Kaur**[*], **Jasmeen Visla**[*], **Sidika Karakas**[*], and **Lars Berglund**[*,†]

[*]Department of Internal Medicine, School of Medicine, University of California, Davis, CA, USA

[†]Department of Veterans Affairs, Northern California Health Care System, Sacramento, CA, USA

Abstract

Objective—Levels of lipoprotein(a), Lp(a), an independent risk factor for cardiovascular disease (CVD), are affected by sex hormones. Women with polycystic ovary syndrome (PCOS) have elevated androgen levels and are at increased CVD risk. We investigated the impact of PCOS-related hormonal imbalance on Lp(a) levels in relation to apo(a) gene size polymorphism, a major regulator of Lp(a) level.

Design—Cross-sectional

Patients—Forty one Caucasian women with PCOS based on the NIH criteria.

Measurements—1) Apo(a) gene size polymorphism measured as Kringle (K) 4 repeat number; 2) Total plasma Lp(a) level; 3) Allele-specific apo(a) level assessing the amount of Lp(a) carried by an individual apo(a) allele/isoform; 4) Sex hormone levels.

Results—The mean age was 32±6 years and the mean BMI was 35±8 with 66% of women classified as obese (BMI >30kg/m²). LDL cholesterol was borderline high (3.37 mmol/l) and HDL cholesterol was low (1.06 mmol/l). The distribution of Lp(a) level was skewed towards lower levels with a median level of 22.1 nmol/l (IQR: 6.2–66.5 nmol/l). Lp(a) levels were not correlated with age, body weight or BMI. The median allele-specific apo(a) level was 10.6 nmol/l (IQR: 3.1–31.2 nmol/l) and the median apo(a) size was 27 (IQR: 23–30) K4 repeats. Allele-specific apo(a) levels were significantly and inversely correlated with K4 repeats (r=−0.298, *p*=0.007). Neither Lp(a) or allele-specific apo(a) levels were significantly associated with testosterone or dehydroepiandrosterone sulfate levels.

Conclusions—The apo(a) genetic variability remains the major regulator of plasma Lp(a) levels in women with PCOS.

Keywords

Lp(a); apo(a) isoform; allele-specific apo(a) level; PCOS; sex hormones

Correspondence: Lars Berglund, MD, PhD, Department of Medicine, University of California, Davis, UCD Medical Center, CTSC, 2921 Stockton Blvd, Suite 1400, Sacramento, CA 95817, USA, Phone: (916) 703-9120, Fax: (916) 703-9124, lberglund@ucdavis.edu.

INTRODUCTION

In the general population, an elevated level of plasma lipoprotein(a), Lp(a), is an independent causal risk factor for cardiovascular disease (CVD).[1–4] Screening for elevated Lp(a) levels has been recommended in clinical guidelines for individuals at moderate to high cardiovascular risk.[5] Similar to a low-density lipoprotein (LDL) particle, Lp(a) contains a cholesteryl ester-rich lipid core and one molecule of apolipoprotein (apo) B-100, in addition to a unique, highly glycosylated apolipoprotein, i.e., apo(a), containing repeated loop structures so called Kringles (K).[6] Two different types of K repeats (K4 and K5) are present in the apo(a) gene, of which K4 is further diversified into 10 different subtypes (K4 type 1 through type 10). Of note, K4 type 2 is repeated in variable numbers ranging from 3 to more than 30 copies resulting in an extensive size heterogeneity of the apo(a) gene.[7–9] This apo(a) gene size polymorphism is a major regulator of Lp(a) levels. In general, smaller apo(a) sizes with a fewer number of K4 repeats are associated with a higher plasma level of Lp(a).[9] Further, an association between small size apo(a) and increased CVD risk has been confirmed in a recent meta-analysis.[10] Beyond total plasma Lp(a) level, the allele or isoform-specific apo(a) level, assessing the amount of Lp(a) carried by a defined apo(a) allele, has been useful in predicting Lp(a)-associated CVD risk.[11]

Polycystic ovary syndrome (PCOS) is the most common endocrine disease in young women, affecting 6 to 10% of women of reproductive age.[12] PCOS is characterized by hyperandrogenism, ovarian dysfunction, polycystic ovarian morphology and hyperinsulinemia.[12] Notably, women with PCOS are at significantly increased risk for CVD, and many traditional cardiovascular risk factors have been reported to be elevated in PCOS women compared to non-PCOS women.[12] Although some emerging CVD risk markers are reported to be elevated in PCOS women, little is known about Lp(a). Sex hormones are among the few non-genetic factors that possess the potential to regulate Lp(a) levels[13, 14] and are dysregulated in PCOS. Unlike the situation in the general population, the role of Lp(a) in the development of CVD in women with PCOS remains elusive. Only a few studies have investigated Lp(a) levels in PCOS women[15–18] and most have reported elevations in concentrations. However, none of these studies have considered apo(a) size polymorphism, despite its major role in Lp(a) regulation. In the present study, we investigated the relationship between Lp(a) and sex hormone levels in relation to apo(a) gene size polymorphism in women with PCOS. To minimize an impact of ethnicity/race, a well-recognized strong modulator of Lp(a) levels, we have focused our studies on Caucasian women.

MATERIALS AND METHODS

Human subjects

The study subjects were recruited from a PCOS population followed at the University of California (UC) Davis Medical Center, Davis, CA. The diagnosis of PCOS was made according to the NIH criteria. Subjects had not used oral contraceptives, steroid hormones, or any other treatments affecting ovarian function, insulin sensitivity, or lipid profile for at least three months prior to the study. The present study was based on the findings of 41

Caucasian women. The study was approved by the Institutional Review Board at the UC Davis and informed consent was obtained from all subjects.

Clinical and biochemical assessments

Body mass index (BMI) was calculated as body weight (kg) divided by squares of height (m^2). Participants were asked to fast for 12 hours and blood samples were drawn into tubes containing EDTA. Plasma samples were separated and stored at −80°C prior to analysis. Concentrations of triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, apoB-100, glucose, insulin, hemoglobin A1C (HbA1c), steroid hormones [testosterone, dehydroepiandrosterone sulfate (DHEA-S), and cortisol] and high sensitivity C-reactive protein (CRP) were determined using standard procedures. LDL-C levels were calculated in subjects with triglyceride levels of <400 mg/dL with the formula of Friedewald.[19] Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated as previously described.[20] Plasma Lp(a) concentrations were measured by an apo(a) size insensitive sandwich enzyme-linked immunosorbent assay (Mercodia Inc, Uppsala, Sweden).[21, 22] The interassay coefficient of variation was <5%.

Determinations of apo(a) isoform size and allele-specific apo(a) levels

Apo(a) isoform sizes were determined by Western blotting technique with SDS-agarose gel electrophoresis of plasma samples, followed by immonoblotting as previously described.[7, 23] Protein dominance was determined by optical analysis of the apo(a) protein bands on Western blot, followed by a validation by computerized scanning. To determine allele-specific apo(a) levels, for each of the apo(a) protein bands, Lp(a) levels were apportioned according to the degree of intensity of the bands on the Western blot as previously described.[23]

Statistics

Statistical analysis was performed with SPSS software (SPSS Inc, Chicago, IL). Results were expressed as mean ± standard deviation (SD) for normally distributed variables, or median with interquartile range for non-normally distributed variables. Triglyceride levels were logarithmically transformed, and Lp(a) and allele-specific apo(a) levels were square root transformed to achieve normal distributions. Both the larger and smaller apo(a) isoforms of subjects with two distinguishable bands and one isoform of subjects with a single band were considered for statistical analyses. Pearson's correlation coefficients were calculated to describe the magnitude and direction of the association of Lp(a) or allele-specific apo(a) levels with other variables. Group means were compared using Student's *t*-test. All analyses were two-tailed, and *p*-values less than 0.05 were considered statistically significant.

RESULTS

Clinical characteristics of study population

The mean age of PCOS women was 32±6 years (Table 1). The mean BMI was 35±8 with 66% of women classified as obese (BMI >30 kg/m^2). The mean LDL cholesterol concentration was borderline high (3.37 ± 0.78 mmol/l) and 46% of women had LDL-C

levels above 3.37 mmol/l (130 mg/dl). The mean HDL cholesterol concentration was low (1.06 ± 0.18 mmol/l) and the prevalence of a low HDL-C concentration (<1.29 mmol/l or <50 mg/dl) was 90%. Total cholesterol, ApoB-100, triglyceride, HbA1c, glucose and cortisol concentrations were within normal range. The median insulin concentration was 108 pmol/l and 41% of women had an elevated concentration above 120 pmol/l. The prevalence of insulin resistance (HOMA-IR >3.0) was 66%. The mean testosterone concentration was high (2.98 ± 0.97 nmol/l) with 78% of women having an elevated level above 2.08 nmol/l (0.60 ng/ml). The mean DHEA-S concentration was 4451 ± 2280 nmol/l, with only 7% of women having an elevated level above 8142 nmol/l (300 μg/dl). Furthermore, the mean CRP concentration was 6.5 mg/l, twice as high as the commonly used threshold value of 3 mg/l. Of note, 51% of women had an elevated CRP level (>3 mg/l).

Plasma Lp(a) and allele-specific apo(a) levels and apo(a) size polymorphism

As in the general population, the distribution of Lp(a) level was skewed towards lower levels (Figure 1A). Similarly, allele-specific apo(a) level assessed as the amount of Lp(a) carried by a defined apo(a) allele/isoform had a skewed distribution towards lower levels (Figure 1B). The median Lp(a) and allele-specific apo(a) concentration was 22.1 nmol/l (IQR: 6.2–66.5 nmol/l) [9.2 (2.6–27.7) mg/dl] and 10.6 nmol/l (IQR: 3.1–31.2 nmol/l) [4.4 (1.3–13.0) mg/dl], respectively (Table 2). Twenty four % of women (10 out of 41) had elevated Lp(a) level above 72 nmol/l (30 mg/dl), a commonly used cut-off value for high Lp(a) level.

Apo(a) protein isoforms were detected in all subjects, and 95% of women had double protein bands on Western blot. Further, the distributions of apo(a) dominance pattern among subjects expressing double protein bands [i.e., heterozygous for apo(a)] were analyzed. Interestingly, the distribution was fairly comparable between different apo(a) dominance patterns. Thus, a smaller isoform was dominating in 33% of women and a larger apo(a) was dominating in 36% of women. The presence of co-dominating apo(a) isoforms, as determined by a similar expression level of apo(a) protein bands on Western blot, was 31%. The median apo(a) size determined by K4 repeats was 27 (IQR: 23 and 30). In addition, the prevalence of small size apo(a) (22 K4 repeats) associated with increased risk for CVD, was 21% (Table 2).

Correlations of Lp(a) and allele-specific apo(a) levels with apo(a) size polymorphism and other clinical variables

As expected, the Lp(a) level was not correlated with a subject's age, body weight or BMI. Analyses across high and low BMI groups (above or less than 30 kg/m^2) did not result in any significant correlations between these variables. Furthermore, there were no significant correlations between the levels of Lp(a) and other plasma lipid and lipoproteins, including total cholesterol (r=0.153, p>0.05), LDL cholesterol (r=0.123, p>0.05), HDL cholesterol (r=0.083, p>0.05), triglyceride (r=0.155, p>0.05) and apoB-100 (r=0.132, p>0.05). Additionally, we did not find any significant correlations between the levels of Lp(a) and CRP (r=0.071, p>0.05), insulin (r=0.056, p>0.05) or HOMA-IR (r=−0.195, p>0.05).

As sex hormones have been shown to impact plasma Lp(a) level, we next investigated the relationship between Lp(a) and sex hormone levels. No significant correlations were found

between Lp(a) and testosterone (r=−0.045, *p*>0.05) or DHEA-S (r=−0.129, *p*>0.05) levels. Further analyses stratified by high and low testosterone (above and below 2.08 nmol/l) or high and low DHEA-S (above and below 8142 nmol/l) levels failed to alter the significance of these associations. In addition, Lp(a) level was not correlated with cortisol level (r=0.100, *p*>0.05).

As in the general population, allele-specific apo(a) levels were significantly and inversely associated with K4 repeat numbers (r=−0.298, *p*=0.007) (Figure 1C). Similar to the findings of Lp(a) levels, allele-specific apo(a) levels were not correlated with clinical and biochemical measurements (age, body weight, BMI, CRP, insulin, HOMA-IR and lipids and lipoprotein levels) as well as testosterone (r=−0.071, *p*>0.05) and DHEA-S (r=−0.125, *p*>0.05) levels. In order to explore the potential modulation of the relationship between allele-specific apo(a) levels and sex hormones by the apo(a) gene size polymorphism, we performed the same correlation analyses by dividing subjects into two groups using the median apo(a) size (i.e., 27 K4 repeats). The findings of these analyses confirmed the previous findings, as we did not find any significant correlations between Lp(a) and hormone levels within each apo(a) size group (above or below 27 K4 repeats).

Additional analysis using multiple linear regression models was performed to explore independent predictors of allele-specific apo(a) levels. When age, BMI, apo(a) size, sex hormones (testosterone and DHEA-S) and CRP were considered in the model, only apo(a) size was independently and strongly associated with allele-specific apo(a) level (β=−0.334, *p*=0.003).

DISCUSSION

This is the first study examining total plasma Lp(a) levels, allele-specific apo(a) levels, and genetic variability of the apo(a) gene in relation to sex hormone levels in women with PCOS. Consistent with studies in other populations,[11] distributions of Lp(a) and allele-specific apo(a) levels in PCOS women were skewed towards lower levels. Further, allele-specific apo(a) levels were significantly and inversely associated with the apo(a) gene size polymorphism as determined by the number of K4 repeats. The median Lp(a) concentration as well as the prevalence of an elevated Lp(a) level in PCOS women were comparable to that previously reported observations in Caucasian women without PCOS.[11] Neither Lp(a) or allele-specific apo(a) levels were significantly impacted by anthropometric characteristics, such as age, body weight or BMI, as well as other lipid and lipoprotein levels. Notably, there were no significant correlations between hormone levels and Lp(a) or allele-specific apo(a) levels. A multivariate regression analysis showed that only apo(a) size was a significant predictor of allele-specific apo(a) levels.

Studies have reported that PCOS women are at increased risk of CVD compared to non-PCOS women. In a recent meta-analysis, women with PCOS had significantly increased concentrations of CRP, homocysteine, plasminogen activator inhibitor-1 activity, vascular endothelial growth factor, asymmetric dimethylarginine, advanced glycation end-products, and Lp(a) compared with controls.[24] A recent consensus statement by the Androgen Excess and Polycystic Ovary Syndrome Society concluded that PCOS women with obesity,

cigarette smoking, dyslipidemia, hypertension, impaired glucose tolerance, and subclinical vascular disease are at risk, and those with metabolic syndrome and/or type 2 diabetes mellitus are at high risk for CVD.[12] In the current study, several lipid and non-lipid CVD risk markers were commonly present, including elevated concentrations of LDL cholesterol, CRP and decreased concentrations of HDL cholesterol. Obesity was common among PCOS women with two thirds having BMI levels >35 kg/m^2. In this context of a risk environment, it was of particular interest to assess any specific contribution of Lp(a).

Lp(a) levels are under a strong genetic regulation and are not affected by changes in lifestyle and physical characteristics, as well as currently available lipid-lowering drugs. However, sex hormones constitute an important exception and studies have shown menopause-related changes in Lp(a) concentration and Lp(a)-lowering effect by testosterone administration and Lp(a)-increasing effect by orchidectomy in men,[25, 26] or hormone-replacement therapy in women.[14, 27–32] In view of this, a better understanding of the role of Lp(a) in PCOS has attracted attention. One study in Turkish women reported a higher mean Lp(a) level in PCOS women compared to healthy controls (25.2 mg/dl vs. 16.7 mg/dl, respectively).[15] Also in this study, obese PCOS women had higher mean Lp(a) levels compared to non-obese PCOS women, a finding that we could not replicate. In the Turkish study, PCOS women were younger and less obese than our PCOS women. In another study among Turkish women with PCOS, there was no significant difference in Lp(a) concentrations between cases and controls [16] In another report, mean Lp(a) levels were elevated in women with vs. without PCOS (24 vs. 5.2 mg/dl, respectively).[18] In line with our findings, one quarter (24%) of PCOS women had elevated Lp(a) levels in this study.[18] Further, the Lp(a) concentration was weakly correlated with HDL cholesterol levels and 36% of patients with PCOS with an otherwise normal plasma lipid profile showed elevated levels of Lp(a), apoB or small, dense LDL. In a Mediterranean cohort, mean Lp(a) levels were 6 mg/dl, 21 mg/dl, and 40 mg/dl in control, ovulatory and anovulatory PCOS women, respectively.[33] Consistent with our findings, Lp(a) levels were not correlated with insulin concentrations or HOMA-IR.

Neither Lp(a) or allele-specific apo(a) levels correlated significantly with testosterone levels. This finding is in line with previous observations reporting no correlation between testosterone and Lp(a) levels.[33] It is tempting to speculate that other factors, such as a high risk metabolic environment with an increased inflammatory burden, may impact on the relationship between sex hormones and Lp(a) in PCOS. Indeed, our PCOS women had a high level of inflammation as detected by CRP concentrations twice as high as the generally accepted cut-off point for elevated levels (6.5 mg/l vs. 3.0 mg/l). In the general population, we have previously shown that subjects with a high burden of systemic inflammation exhibited elevated Lp(a) and allele-specific apo(a) levels, in particular, those carried by atherogenic smaller apo(a) sizes.[34–36] Therefore, we cannot rule out that the PCOS-induced high inflammatory environment could have contributed to modulate any effect of sex hormones on Lp(a) concentrations.

Lack of assessment of apo(a) gene size polymorphism in previous studies, makes it difficult to exclude that differences in apo(a) size distributions could contribute to any observed elevations in Lp(a) levels in PCOS women. The determination of apo(a) size polymorphism allowed us to characterize the distributions of apo(a) isoform sizes and dominance patterns,

allele-specific apo(a) level, a level associated with a defined apo(a) size, as well as the prevalence of small atherogenic apo(a) isoforms to an extent not previously done in a PCOS population. Use of allele-specific apo(a) levels, taking both the genotypic and phenotypic characteristics of Lp(a) into account, has shown a greater predictive power for coronary heart disease (CHD) risk assessment.[11]

In the current study, apo(a) protein isoforms were detected in all subjects, with 95% of women being heterozygotes and the overall pattern for apo(a) dominance in this PCOS population differed from those in other populations (Figure 2). The distribution of apo(a) dominance pattern in PCOS women was 33%, 36% and 31% for the smaller, larger and co-dominating apo(a) isoforms within any given individual (i.e., a fairly even distribution). In contrast, in our previous studies among Caucasian women scheduled for coronary angiography, the smaller apo(a) isoform was dominating in the majority of women (63%).[23] Our data in Caucasian women recruited from the general population (i.e., healthy women) indicated that smaller apo(a) isoform was dominating in more than half of women (56%) (Figure 2). Although the clinical relevance of this differential distribution in apo(a) dominance between women with PCOS, women at risk for CHD and healthy women remains to be clarified, it is tempting to speculate that the lower frequency of smaller apo(a) isoform sizes in PCOS women may influence the degree of cardiovascular risk due to Lp(a). However, further studies are needed to determine the basis for the apo(a) dominance pattern as well as its relation to PCOS-associated CHD risk. The frequency of small size apo(a) (22 K4 repeats) was 21%, comparable to previously reported frequency in non-PCOS populations.[11, 37] Of note, a meta-analysis indicated a two-fold increased risk of CHD in subjects carrying smaller apo(a) isoforms vs. subjects carrying larger apo(a) isoforms.[10]

We acknowledge some limitations in the current study. We recruited Caucasian women and further studies in other racial/ethnic groups as well as in other settings are needed to address the generalizibilty of the findings. Although genotyping of apo(a) was not feasible, apo(a) phenotyping was successfully done in each subject, allowing accurate determination of apo(a) gene size polymorphism as well as allele-specific apo(a) level. Although we did not include a healthy matched control (i.e., non-PCOS) group in the study, availability of abundant information from previous studies in PCOS and other populations, including ours, with regard to Lp(a) level, allele-specific apo(a) level and apo(a) size heterogeneity, as well as on the role of sex hormones in Lp(a) regulation, has enabled us to place our findings in a rich context. However, the findings of this study should be interpreted with caution and extended to studies with a larger sample size, including appropriate control groups. Importantly, future long-term prospective studies in multiethnic populations with data on apo(a) sizes and clinical outcomes will shed light on the role Lp(a) and apo(a) genetic variability in PCOS-associated increased CVD risk.

In conclusion, although our findings need validation in larger studies, our results emphasize the major role of genetic variability of the apo(a) gene in regulating plasma levels of Lp(a) in women with PCOS. Further studies are needed to shed lights into the complex interplay between genetic and non-genetic factors in regulating Lp(a) levels and in modulating the well-established relationship between elevated Lp(a) levels and CVD risk in conditions associated with hormonal imbalance such as PCOS.

Acknowledgments

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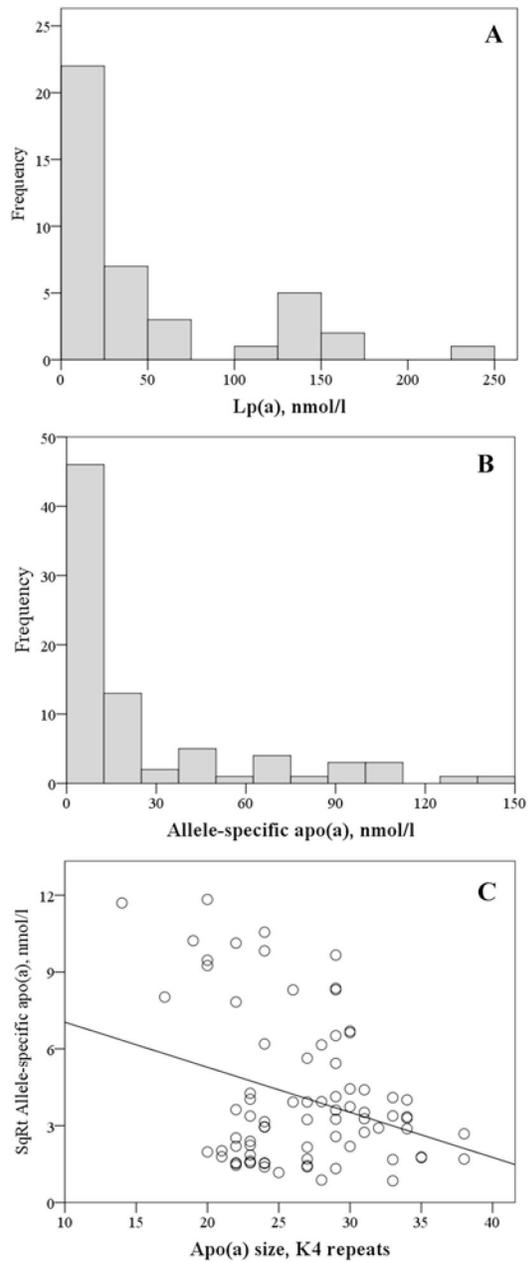
Figure 1. Distributions of Lp(a) levels (A), allele-specific apo(a) levels (B) and correlations of allele-specific apo(a) levels with apo(a) size polymorphism (C) in PCOS women
Allele-specific apo(a) level was determined as described in the Materials and Methods section. More information on the determination of allele-specific apo(a) levels can be found in reference #23. Pearson's correlation analysis with square-root transformed allele-specific apo(a) level was used to describe the relationship with apo(a) size polymorphism.
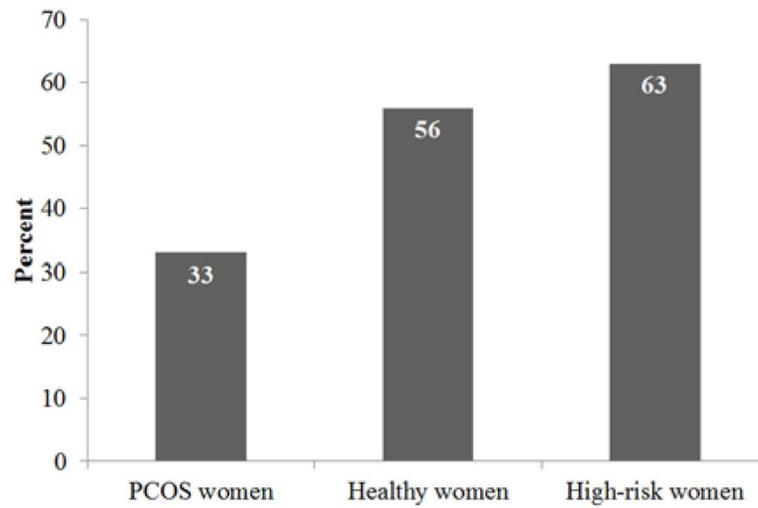
Figure 2. Distribution of smaller apo(a) dominance pattern in PCOS women vs. women at risk for CHD or healthy women

Data for women at risk for CHD was from reference #23. Data for healthy women was based on unpublished observation from our laboratory. Apo(a) dominance pattern was assessed as described in the Materials and Methods section.

Table 1

Clinical characteristics of PCOS women

Characteristics	
Age (years)	32 ± 6
Weight (kg)	96 ± 24
Body mass index (kg/m^2)	35 ± 8
Normal weight (<25 kg/m^2)	5 (12%)
Overweight (25–30 kg/m^2)	9 (22%)
Obese (>30 kg/m^2)	27 (66%)
Total cholesterol (mmol/l)	4.97 ± 0.91
LDL cholesterol (mmol/l)	3.37 ± 0.78
Elevated LDL cholesterol (>3.37 mmol/l), n (%)	19 (46%)
HDL cholesterol (mmol/l)	1.06 ± 0.18
Low HDL cholesterol (<1.29 mmol/l), n (%)	37 (90%)
Triglycerides (mmol/l)	1.05 (0.85–1.64)
Apolipoprotein B-100 (g/l)	0.80 ± 0.22
C-reactive protein (mg/l)	6.5 ± 7.3
Elevated C-reactive protein (>3 mg/l), n (%)	21 (51%)
Testosterone (nmol/l)	2.98 ± 0.97
Elevated testosterone (>2.08 nmol/l), n (%)	32 (78%)
DHEA-S (nmol/l)	4451 ± 2280
Elevated DHEA-S (>8142 nmol/l), n (%)	3 (17%)
Cortisol (nmol/l)	326 ± 152
Hemoglobin A1c (DCCT %)	5.6 ± 0.4
Hemoglobin A1c (mmol/mol)	37.3±4.8
Glucose (mmol/l)	5.60 ± 0.83
Insulin (pmol/l)	108 (60–150)
HOMA-IR	4.3 ± 2.4

Data are shown as mean ± SD or median (IQR).

Abbreviations: DHEA-S, dehydroepiandrosterone sulfate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA-IR, homeostasis model of insulin resistance

Table 2

Lipoprotein(a) and allele-specific apo(a) levels and apo(a) size

	Lp(a) (nmol/l)	Allele-specific apo(a) (nmol/l)	Apo(a) size (K4 repeats)
Median (IQR)	22.1 (6.2–66.5)	10.6 (3.1–31.2)	27 (23–30)
Minimum	3.8	0.7	14
Maximum	233.5	140.2	38
Prevalence of high Lp(a) (>72 nmol/l) or small size apo(a) (22 K4 repeats) [*]	10 (24%)	-	17 (21%)

[*] Based on both apo(a) alleles of heterozygotes with two protein bands expressed on Western blot (n=39) and one isoform of persons with only a single expressed band on Western blot (n=2). Thus, the total number of isoforms detected was n=80.