Searching for a common mechanism for placenta-mediated pregnancy complications and cardiovascular disease: role of lipoprotein(a)

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Objective: To investigate lipoprotein(a) [Lp(a)], a well known cardiovascular risk factor, in women with history of placenta-mediated pregnancy complications (PMPC) compared with healthy uneventful-pregnancy women (HW), and the role of LPA gene functional polymorphisms in modulating both Lp(a) levels and PMPC risk.
Design: Retrospective observational study.
Setting: University hospital.
Patient(s): A total of 360 women with history of PMPC (154 preeclampsia [PE], 121 stillbirth [SB], and 85 small for gestational age [SGA]) and 270 HW.
Intervention(s): Not applicable.
Main Outcome Measure(s): Lp(a) levels measurement and LPA +93C >T and +121G>A polymorphisms genotyping.
Result(s): In PMPCs we observed higher Lp(a) levels than those found in HW and an association with PMPC risk, also after adjustment for age, familial history of cardiovascular disease, and traditional risk factors. By analyzing Lp(a) concentrations according to each pregnancy complication, we observed significantly higher Lp(a) levels in women with history of SB and PE, conferring 2.5-fold and 2-fold increased risks, respectively; no association with SGA was observed. Lp(a) concentrations progressively and significantly increased as LPA unfavorable allelic burden increased; unfavorable allelic burden influenced SB and PE risk.
Conclusion(s): We evidenced, for the first time, an association between high Lp(a) concentrations and history of SB, and we confirmed the role of Lp(a) in PE risk; this well known atherothrombotic marker might represent one of the possible mechanisms shared by PMPC and cardiovascular disease. (Fertil Steril® 2016;105: 1287–93. ©2016 by American Society for Reproductive Medicine.)
Key Words: Lipoprotein(a), stillbirth, preeclampsia, LPA gene

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Data from the literature provides evidence that a history of placenta-mediated pregnancy complications (PMPC), such as preeclampsia (PE), small-for-gestational-age neonate (SGA), and stillbirth (SB), increases the risk of cardiovascular disease (CVD) later in life (1, 2), and it is a major risk factor for CVD in the American Heart Association (AHA)/American Stroke Association (ASA) guidelines (3, 4).

There is a common pathophysiologic pathway of endothelial dysfunction linking placental and vascular disorders. Beyond traditional cardiovascular risk factors, there is a wide variety of cardiovascular biomarkers endothelial dysfunction-related, which are still not widely explored in the clinical studies, such as lipoprotein(a) [Lp(a)]. Lipoprotein(a) is a plasma lipoprotein composed of a low-density lipoprotein (LDL) particle and an additional lipoprotein, apolipoprotein(a), linked to apoB-100 of the LDL (5). Lp(a) plays a relevant role in the development of atherosclerosis, through multiple pathways, such as Lp(a)-derived cholesterol entrapment in the intima, inflammatory cell recruitment, and binding of

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proinflammatory-oxidized phospholipids to endothelial cells (6, 7). Lp(a) also contributes to a prothrombotic phenotype through antifibrinolytic actions (8), owing to its high homology to plasminogen and to the inhibition of tissue factor pathway inhibitor.

Data from literature provide evidence that elevated Lp(a) concentrations represent an independent risk factor for premature cardiovascular disease (9–11).

Lp(a) concentrations are under genetic control at the concentration of biosynthesis of the apo(a) protein, which is encoded by the LPA gene locus; allelic differences at LPA locus may be responsible for the variations in Lp(a) phenotype (12). Variants at LPA locus have been associated with both an increased levels of Lp(a) and an increased risk of coronary disease (11).

The role of Lp(a) in pregnancy complications has been the object of some clinical studies, which report indefinite results (13). Few studies evaluate the relationship between Lp(a) and PE (14, 15), only one study explored its role in affecting SGA predisposition (15), but, to the best of our knowledge, no study is available concerning the influence of Lp(a) in modulating SB risk.

In this scenario, we investigated Lp(a) in women with history of SB, PE, or SGA to evaluate the role of this well known atherothrombotic marker in obstetrical negative outcome risk; we also investigated the role of LPA functional polymorphisms in modulating both Lp(a) levels and PMPC risk.

MATERIALS AND METHODS

Study Population

The entire study population comprised 870 consecutive women referred to the Gender Medicine Clinic of the Center for Atherothrombotic Disease, Department of Experimental and Clinical Medicine, University Hospital, Florence, from 2010 to 2013, to be assessed for vascular risk.

In Supplemental Figure 1 (available online at www.fertstert.org) the study population is reported. Three hundred sixty women with history of pregnancy complications related to uteroplacental vascular insufficiency (PMPC) (154 with history of PE, 121 SB, and 85 SGA) were referred from gynecologists to the gender medicine clinic to be assessed for their cardiovascular risk, because history of PMPC represents a new cardiovascular risk factor; information concerning the adverse obstetrical outcomes derived from written gynecologists' clinical reports (PE defined as systolic blood pressure >140 mm Hg or diastolic blood pressure ≥ 90 mm Hg and 24-hour proteinuria ≥ 0.3 g; SB defined as late intrauterine fetal death after 24 completed weeks of pregnancy; SGA defined as infant born with a birth weight less than the 10th percentile, according to Royal College of Obstetricians and Gynaecologists guidelines).

Two hundred seventy healthy women with no history of vascular disease, referred to Gender Medicine Clinic for evaluating thrombotic risk before taking estrogen-progesterone therapy or because of family history of vascular disorders were considered as control subjects. These women delivered after uneventful pregnancy. To identify disease-free control subjects, and to exclude women who were thought to have any form of vascular disease, a detailed interview addressing personal and familial history was performed. All women were investigated for Lp(a) after a minimum of 12 weeks after birth. None were pregnant or had used oral contraceptives within 8 weeks before testing.

Exclusion criteria were the presence of diabetes mellitus, renal failure, pregnancy complications explained by anatomic, chromosomal, endocrine, or immunologic abnormalities, or intercurrent infectious events. Non-white ethnicity (primarily because of the difference in the prevalence of the genetic polymorphisms) represented a further exclusion criterion. Finally, women who refused to assent to the genetic analysis were not included.

Informed written consent for anonymous data analysis was obtained from each woman, and the study was approved by the local Ethical Review Board. The investigation conformed with the principles outlined in the Declaration of Helsinki.

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes with the use of GeneCatcher gDNA Blood Kit (Invitrogen) with the aid of the automated platform Freedom EVO 150 (Tecan).

LPA +93C>T (rs1853021) and +121G>A (rs1800769) polymorphisms were detected with the use of polymerase chain reaction (PCR)–restriction fragment length polymorphism analysis. The sequence surrounding the two SNPs was amplified by means of PCR reaction with the following settings: one denaturation cycle at 95°C for 5 minutes, 35 cycles with denaturation at 94°C for 1 minute, annealing at 54°C for 50 seconds, and extension at 72°C for 50 seconds, followed by a final extension at 72°C for 7 minutes. The reaction was performed in a final volume of 25 μL with 100 ng of genomic DNA, 0.2 mmol/L of each dNTP, 1 μL of a 10 μmol/L forward primer (5′-TGACATTGCACTCTCAAATATTTT-3′), 1 μL of a 10 μmol/L reverse primer (5′- AGAACCACTTCC TTATGTTCCA-3′), and 0.5 U of Taq polymerase (GoTaq; Promega Italia) in 1× PCR Buffer.

To detect LPA +93C>T SNP, 10 μL of the PCR products (222 bp) were subjected to digestion with TaiI restriction enzyme (Fermentas International), whereas the evaluation of LPA +121G>A transition requires an enzymatic digestion with SduI (Fermentas International). Both the reactions are carried out at 37°C for 16 hours, and the digestion fragments were separated on 3.5% agarose gel.

Lipoprotein(a) Measurements

Blood samples were collected from the antecubital vein into evacuated plastic tubes (Vacutainer) after an overnight fast. Serum samples were obtained by centrifuging blood collected in evacuated tubes without anticoagulant at 2,000*g* for 10 minutes at 4°C and subsequently stored at −20°C. Lp(a) levels were detected by means of an immune-nephelometry method on serum samples with the use of the LPAX reagent in conjunction with Image800 Immunochemistry Systems and Lipoprotein(a)

Calibrator (Beckman Coulter). The cutoff for this analyte is represented by values equal to 300 mg/L, which represents an independent risk factor for vascular disease (16, 17). Intra-assay and inter-assay coefficients of variation were <5%.

Statistical Analysis

Few data are available regarding Lp(a) levels in women with history of obstetrical events (14); studies have documented a prevalence of high Lp(a) levels of ~30% and ~10% in women with history of obstetrical events and uneventful pregnancy, respectively. Based on this observation, a sample size of ≥100 women for each group was deemed to be sufficient to prove/exclude an association between high Lp(a) levels and PMPC with a statistical power of 90% (β) and a significance value of 5% (α).

Statistical analyses were performed with the use of the SPSS software for Windows (Version 11.5).

Age was expressed as median (range), and categoric variables were expressed as n (%). The only continuous variable (age), which showed a normal distribution, was analyzed by means of a parametric test (Student *t* test); the nonparametric Mann-Whitney test for unpaired data was used for comparisons of the other continuous variables between single groups. Chi-square test was used to test for proportions and for deviation of genotype distribution of LPA polymorphisms from the Hardy-Weinberg equilibrium. Because Lp(a) distribution was right-skewed, values were log-transformed in regression analyses and back-transformed for data presentation. A logistic regression analysis was used to evaluate the role of Lp(a) levels in modulating pregnancy complications risk. Variables which showed at univariate analysis a significant association with the disease were introduced into the multivariate model, as well as age and smoking habits. During multivariate analysis, a first model (model 1) was created by adjusting for age and familial history of cardiovascular disease; subsequently, a second model (model 2) was created by also adjusting for hypertension, dyslipidemia, smoking habit, and body mass index (BMI) >25 kg/m^2. Odds ratios (ORs) and 95% confidence intervals (CIs) are presented. A *P* value of <.05 was considered to indicate statistical significance.

To investigate the relationship between Lp(a) concentrations and LPA functional alleles, the study population was divided into five subgroups according to allelic burden: group 0, women homozygous for LPA 120GG and 93TT; group 1, women carrying one LPA functional variant related to increased gene expression; group 2, women carrying two LPA functional variants related to increased gene expression), group 3, women carrying three LPA functional variants related to increased gene expression; and group 4, women homozygous for LPA 120AA and 93CC. Kruskall-Wallis test was performed to compare Lp(a) concentrations among different groups of allelic burden.

To evaluate the influence of allelic burden on pregnancy complications risk, a linear regression analysis was performed and results expressed as regression coefficient (β) ± SE.

RESULTS

Lipoprotein(a) Concentrations and Placenta-mediated Pregnancy Complications

Demographic, clinical, and laboratory characteristics of the study population are reported in Table 1. A higher prevalence of hypertension, BMI >25 kg/m^2, dyslipidemia, and familial history of cardiovascular disease was observed in women with history of PMPC compared with control subjects. When analyzing traditional cardiovascular risk factors according to each pregnancy complication, we observed a

TABLE 1

Demographic and clinical characteristics of the study population.

Characteristic	Patients (n = 360)	Control subjects (n = 270)	*P* Value
Age, y	35 (19–49)	34 (22–40)	.7
Pregnancy complications			
Stillbirth	121 (33.6%)	—	
Preeclampsia	154 (42.8%)	—	
Small-for-gestational-age neonate	85 (23.6%)	—	
Cardiovascular risk factors			
Smoking habits	59 (16.4%)	46 (17.0%)	.8
Hypertension	36 (10.0%)	7 (2.6%)	.0003
BMI >25 kg/m^2	76 (21.0%)	38 (14.1%)	.02
Dyslipidemia	66 (18.3%)	15 (5.6%)	<.0001
Total-C, mg/dL	204.3 ± 51.0	187.4 ± 37.0	.003
LDL-C, mg/dL	115.6 ± 33.9	102.4 ± 31.6	<.0001
HDL-C, mg/dL	62.4 ± 18.0	67.4 ± 14.7	.002
TG, mg/dL	114.8 ± 53.2	88.9 ± 48.8	.008
Lp(a) mg/L	285.9 (236.5–335.4)	185.1 (159.4–210.9)	.03
Lp(a) >300 mg/L	115 (31.9%)	46 (17.1%)	<.0001
Family history of CVD	96 (26.6%)	27 (10%)	<.0001

Note: Values are presented as geometric mean (range), n (%), or median ± SD. BMI = body mass index; CVD = cardiovascular disease; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; Lp(a) = lipoprotein(a); TG = triglycerides; Total-C = total cholesterol.

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significantly higher prevalence of hypertension and BMI >25 kg/m^2 in women with history of SB and PE; regarding lipid profile, a higher percentage of dyslipidemic women in the SB, PE, and SGA groups compared to control women was found (Supplemental Table 1, available online at www.fertstert.org). Lp(a) concentrations were assessed 12–25 weeks after delivery; a significantly higher percentage of women with history of PMPC (31.9%) exhibited Lp(a) concentrations >300 mg/L compared with healthy control subjects (17.1%; $P<.0001$; Table 1). Higher Lp(a) concentrations in both patients and control subjects with familial history of CVD (median 338.2 mg/L [range 237.2–440.1] and 205.9 mg/L [138.3–273.7], respectively) compared with patients and control subjects without familial history of CVD (253.4 mg/L [199.3–307.5] and 182.2 mg/L [154.2–210.2], respectively) were found.

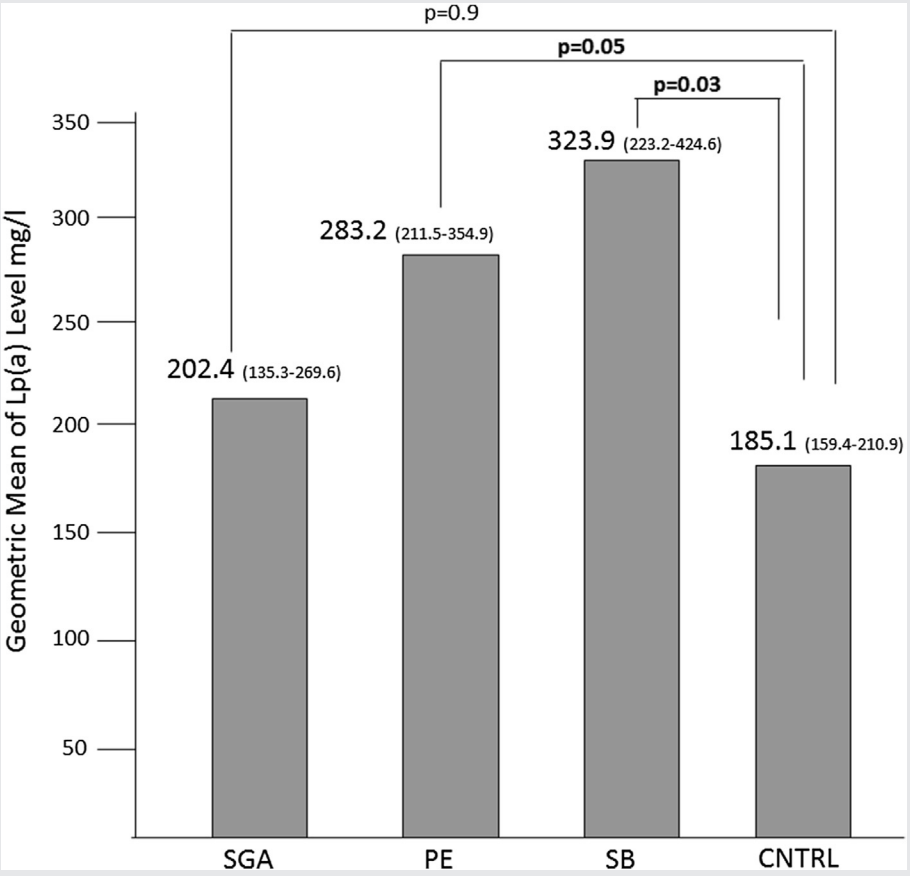
Patients with pregnancy complications were more likely to have cardiovascular risk factors, which may represent confounders; therefore, we performed an additional analysis by excluding potential confounders, such as smoking, chronic hypertension, BMI >30 kg/m^2 dyslipidemia, and familial history of CVD; our results showed a significantly higher Lp(a) concentrations in women with history of PMPC compared with control subjects (Supplemental Table 2, available online at www.fertstert.org).

When we analyzed Lp(a) concentrations according to each pregnancy complication (SGA, PE, SB), we observed significantly higher Lp(a) levels in women with history of SB and PE, whereas in women with history of SGA Lp(a) levels were higher, though not significantly, compared with those found in control subjects (Fig. 1).

To search for a possible association between high Lp(a) levels and PMPC, we performed a logistic regression analysis, which showed a significant association between Lp(a) concentrations and obstetrical negative outcomes (OR 1.93 [95% CI 1.20–3.09]; $P=.006$); after adjustment for age and familial history of cardiovascular disease (model 1), as well as for hypertension, smoking habit, BMI, dyslipidemia and timing from delivery (model 2), high Lp(a) concentrations remained significantly associated with PMPC (Table 2); in particular, high Lp(a) levels conferred a 2.5-fold increased risk of SB and a 2-fold increased risk of PE, even after adjustment in model 1 and model 2. No association between high Lp(a) levels and SGA was observed (Table 2).

Because its seems backward to use Lp(a) measured after the obstetrical complications to predict these negative

FIGURE 1



Lipoprotein (a) [Lp(a)] levels according to each placenta-mediated pregnancy complications (PMPC). CNTRL = control; PE = preeclampsia; SB = stillbirth; SGA = small-for-gestational-age neonates.

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TABLE 2

Logistic regression analyses on the association between Lp(a) levels and placenta mediated pregnancy complications.

	Univariate analysis		Multivariate analysis (model 1)		Multivariate analysis (model 2)	
Complication	**OR (95% CI)**	***P* Value**	**OR (95% CI)**	***P* Value**	**OR (95% CI)**	***P* Value**
PMPC (n = 360)	1.93 (1.20–3.09)	.006	1.75 (1.08–2.82)	.02	1.70 (1.02–2.90)	.04
Stillbirth (n = 121)	2.55 (1.29–5.05)	.007	2.36 (1.19–4.66)	.013	2.30 (1.10–4.81)	.02
Preeclampsia (n = 154)	2.43 (1.23–4.78)	.01	2.19 (1.07–4.47)	.03	2.37 (1.12–4.99)	.02
Small-for-gestational-age neonate (n = 85)	0.98 (0.38–2.52)	.9	—		—	

Note: CI = confidence interval; OR = odds ratio.

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outcomes, we performed a supplemental analysis in which Lp(a) concentrations are considered as the outcome. At linear regression analysis we observed a significant influence of a history of PMPC on Lp(a) concentrations ($\beta = 0.11 \pm 0.05$; $P=.04$); in particular, both SB and PE significantly correlated with Lp(a) levels ($\beta = 0.17 \pm 0.07$ [$P=.02$]; $\beta = 0.14 \pm 0.06$ [$P=.02$]; respectively).

LPA Polymorphisms and Lp(a) Concentrations

All subjects were genotyped for two LPA gene polymorphisms (LPA 93C>T and LPA 121G>A); genotype distribution and allele frequencies were in agreement with those predicted by Hardy-Weinberg equilibrium. Regarding the LPA 121G>A polymorphism, women with history of PMPC exhibited a higher, though not significantly, 121A allele frequency, compared with control subjects (0.12 vs. 0.09, respectively); 93T allele frequency was similar between patients and control subjects (0.13 vs. 0.14, respectively).

Owing to the relatively small influence of each polymorphism on Lp(a) phenotype, we evaluated the weight of more than one unfavorable variant in influencing both Lp(a) concentrations and obstetrical complications risk. Accordingly, we divided our study population in relation to allelic burden groups, and we observed that Lp(a) concentrations progressively and significantly increased as allelic burden increased ($P=.001$; Fig. 2).

Regarding the role of allelic burden in modulation of obstetrical complication risk, we performed a further analysis to investigate the role of a single allele or more than one unfavorable alleles in influencing pregnancy negative events; in linear regression analysis, a trend toward a significant influence of allelic burden on PE and stillbirth risk was observed ($\beta = 0.402 \pm 0.065$; $P=.06$).

DISCUSSION

Our findings provided evidence that women with history of pregnancy complicated by SB exhibited high Lp(a) concentrations, beyond a high prevalence of cardiovascular risk factors, such as unfavorable lipid profile, high blood pressure, and BMI >25 kg/m^2. These data could rekindle Lp(a) interest in relation to the history of obstetrical complications; the availability of new drugs that are showing novel therapeutic/safety profiles could lead to introducing preventive strategies in clinical practice.

Data from the literature provide evidence that history of pregnancy complications determines an increased future cardiovascular risk (18, 19); because of its unique cardiovascular and metabolic stress, pregnancy permits the estimation of a woman's lifetime risk. Women who failed this stress test by experiencing placental disorders have an increased future cardiovascular risk, possibly unmasking early or preexisting endothelial dysfunction and vascular disease (20); to date, a history of pregnancy complications is a major risk factor for CVD in the AHA/ASA guidelines (3, 4).

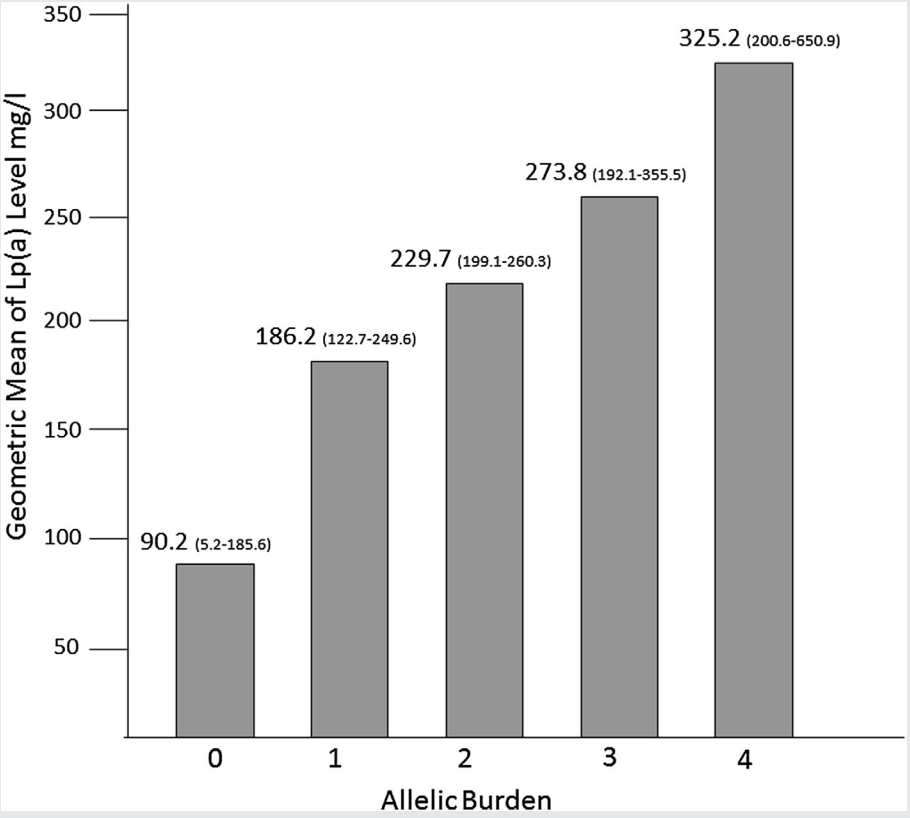
Lipoprotein(a) represents a well known marker of cardiovascular disease (21–23), and it may also be used in risk assessment of subjects in the general population, particularly in intermediate-risk groups (24). During the past years advances have been achieved in understanding its pathophysiologic role (9–11). Elevated Lp(a) concentrations can increase the risk of CVD mainly through prothrombotic/antifibrinolytic effects, because the apo(a) element possesses structural homology with plasminogen, and accelerated atherogenesis as a result of intimal deposition of Lp(a) cholesterol. Interestingly, plasma levels of Lp(a) are similar in both men and women, nevertheless at similarly high levels of plasma Lp(a), women are more likely to experience CVD than men (25, 26).

Data concerning Lp(a) role in pregnancy complications are not clearcut. To date, no previous study had investigated Lp(a) levels in women with history of SB; in the present study we show a role for Lp(a) in modulating SB risk, and this finding may be related to the prothrombotic and antifibrinolytic Lp(a) effects, which could lead to impaired placenta perfusion that can alter oxygen and nutrient supply to the fetus.

We also observed that women with history of PE exhibited high Lp(a) levels, and this finding is in keeping with data by van Pampus et al. (14), but are at variance with those from Manten et al. (15); the higher percentage of control women with high Lp(a) levels (35%) compared with our control group (17%) and the different assay used for Lp(a) determination might explain this discrepancy; several different assays are available to measure Lp(a) levels, and the standardization between Lp(a) assays is still a critical point, which may affect comparisons among studies (25).

Lipoprotein(a) levels are under genetic control, and polymorphisms in the LPA gene have been an object of interest, particularly in coronary artery disease (11) and peripheral

FIGURE 2



Lipoprotein (a) [Lp(a)] lconcentrations according to LPA gene unfavorable alleles burden.

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artery disease (27), as well as in healthy subjects (28). In the present study we investigated the role of rs1853021 and rs1800769 LPA gene polymorphisms in modulating Lp(a) levels, as well as their role in affecting PMPC risk. Our data showed for the first time a progressive and significant increase of Lp(a) concentrations as LPA allelic burden increased, suggesting a relationship between unfavorable allelic burden and negative pregnancy events, particularly SB and PE. Therefore, the present findings, showing a dose-dependent relationship between LPA variants and both Lp(a) levels and risk of pregnancy complications, might support the association between Lp(a) elevated plasma level and negative pregnancy outcomes.

Patients are being followed, and those results will allow us to identify women who will early experience atherothrombotic disease. A limitation of our study is the lack of information concerning KIV repeat number, which better determines Lp(a) concentrations. Also, we are aware that the two polymorphisms investigated in the present study have a relatively small influence on Lp(a) levels; nevertheless we selected these two functional polymorphisms based on others and our previous study (28, 29). Our is a follow-up referral center, at which the most severely affected women as well as healthy women referred for other CVD risk factors would attend, thus representing a further limitation.

Finally, the Lp(a) concentrations evaluated may be related to unresolved pregnancy effects, because the measurements were made in specimens collected 12–25 weeks after delivery; pregnancy-related changes may be not resolved until 6 months after birth; nevertheless, it is well known that Lp(a) increases until 35 weeks of gestation, subsequently decreases slightly until delivery, and thereafter falls to values below early pregnancy concentrations (30).

Our findings highlight the role of Lp(a) in modulating SB and confirm the relationship between Lp(a) and PE risk; interestingly, by analyzing Lp(a) concentrations as the primary outcome, we provided evidence that a history of both SB and PE influenced Lp(a) concentrations, thus strengthening the relationship between this atherothrombotic marker and negative pregnancy outcomes.

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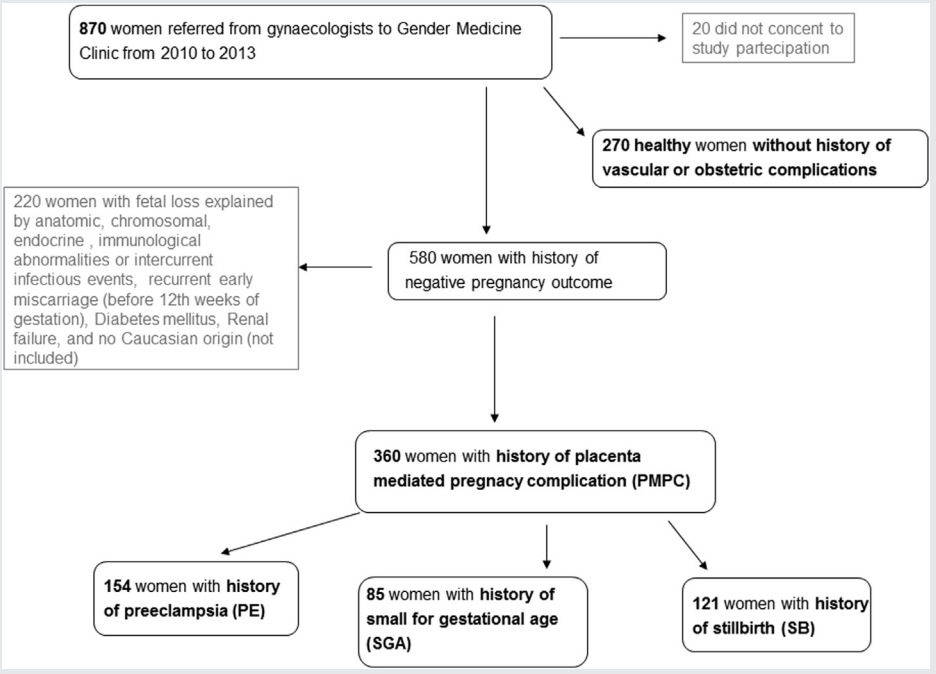
SUPPLEMENTAL FIGURE 1



Flow chart. PE = preeclampsia; SB = stillbirth; SGA = small-for-gestational-age neonates.

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SUPPLEMENTAL TABLE 1

Traditional risk factors according to each subgroup of pregnancy complications compared with uneventful-pregnancy women (control).

Cardiovascular risk factor	Stillbirth (n = 121)	*P* Value	Preeclampsia (n = 154)	*P* Value	Small-for-gestational-age neonate (n = 85)	*P* Value	Control (n = 270)
Smoking habits	25 (20.7%)	.4	22 (14.3%)	.5	12 (14.1%)	.6	46 (17.0%)
Hypertension	8 (6.6%)	.08	23 (14.9%)	< .0001	5 (5.9%)	.2	7 (2.6%)
BMI >25 kg/m^2	28 (23.1%)	.02	40 (25.9%)	.004	8 (9.4%)	.2	38 (14.1%)
Dyslipidemia	21 (17.4%)	.0005	32 (20.8%)	< .0001	13 (15.3%)	.004	15 (5.6%)
Total-C, mg/dL	200.2 ± 52.1	.2	207.1 ± 54.9	.01	203.9 ± 40.7	.02	187.4 ± 37.0
LDL-C, mg/dL	112.5 ± 33.9	.04	118.5 ± 33.4	< .0001	113.9 ± 35.8	.1	102.4 ± 31.6
HDL-C, mg/dL	62.3 ± 16.3	.06	60.3 ± 14.5	.001	67.0 ± 25.6	.5	67.4 ± 14.7
TG, mg/dL	116.7 ± 59.2	.5	144.7 ± 69.7	.02	112.2 ± 52.5	.02	88.9 ± 48.8
Family history of CVD	27 (22.3%)	.001	51 (33.1%)	< .0001	18 (21.2%)	.01	27 (10.0%)

Note: Values are presented as n (%) or median ± SD. *P* values are compared with control. BMI = body mass index; CVD = cardiovascular disease; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TG = triglycerides; Total-C = total cholesterol.

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SUPPLEMENTAL TABLE 2

Demographic and clinical characteristics of the study population after exclusion of women with the potential confounders smoking, chronic hypertension, BMI > 30 kg/m^2, dyslipidemia, and familial history.

Characteristic	Patients (n = 199)	Control subjects (n = 190)	*P* Value
Age	36 (19–46)	34 (22–40)	.8
Pregnancy complications			
Stillbirth	66 (33.2%)	—	
Preeclampsia	81 (40.7%)	—	
Small-for-gestational-age neonate	52 (26.1%)	—	
Cardiovascular risk factors			
Hypertension	13 (6.5%)	1 (0.5%)	.007
BMI >25 kg/m^2	22 (11.1%)	10 (5.3%)	.04
Total-C, mg/dL	179.3 ± 28.7	174.6 ± 26.9	.21
LDL-C, mg/dL	100.7 ± 26.8	93.6 ± 23.7	.06
HDL-C, mg/dL	61.2 ± 16.6	65.3 ± 13.7	.04
TG, mg/dL	90.0 ± 49.9	77.9 ± 35.9	.15
Lp(a) mg/L	249.7 (187.8–311.5)	168.1 (138.3–198.0)	.04
Lp(a) >300 mg/L	58 (29.1%)	28 (14.7%)	.01

Note: Values are presented as geometric mean (range), n (%), or median ± SD. Lp(a) = lipoprotein(a); other abbreviations as in Supplemental Table 1.

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