The 4G/4G Polymorphism of the Hypofibrinolytic Plasminogen Activator Inhibitor Type 1 Gene: An Independent Risk Factor for Serious Pregnancy Complications

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The specific aim of the current study of 133 women with at least 1 pregnancy and measures of hypofibrinolytic and thrombophilic gene mutations was to determine retrospectively whether the mutations were associated with adverse pregnancy outcomes including prematurity, miscarriage, stillbirth, intrauterine growth retardation (IUGR), eclampsia, and abruptio placentae. Four gene mutations (factor V Leiden, methylenetetrahydrofolate reductase [MTHFR], prothrombin, and 4G/5G polymorphism of the plasminogen activator inhibitor type 1 [PAI-1] gene) were assessed by polymerase chain reaction (PCR). One hundred twenty-two women were genotyped for all 4 genes and divided into gene mutation (n = 68) and non–gene (n = 54) groups. The gene mutation group included those with at least 1 thrombophilic mutation (heterozygous for factor V Leiden, heterozygous for prothrombin, and homozygous for MTHFR), or hypofibrinolysis with homozygosity for the 4G polymorphism of the PAI-1 gene. The non–gene mutation group included those with no mutation for all 4 genes (wild-type normal) or who were wild-type normal for the prothrombin and factor V Leiden mutations and heterozygous for MTHFR and/or 4G/5G for the PAI-1 gene, neither heterozygosity associated with coagulation abnormalities. The 68 women with gene mutations, versus 54 in the non–gene mutation group, has more prematurity (10% *v* 4%, $\chi^2 = 5.4$, $P = .021$), more IUGR (3% *v* 0%, $P = .035$), and more total complications of pregnancy (37% *v* 21%, $\chi^2 = 11.6$, $P = .001$). The number of pregnancies ($P = .0001$) and 4G/4G polymorphism of the PAI-1 gene ($P = .029$) were positively associated with complications of pregnancy by stepwise logistic regression when the age, number of pregnancies, and all 4 gene mutations were the explanatory variables. Heritable hypofibrinolysis, mediated by 4G/4G homozygosity for the PAI-1 gene, is an independent significant, potentially reversible risk factor for pregnancy complications, probably acting through thrombotic induction of placental insufficiency.

FAMILIAL THROMBOPHILIA, including the factor V Leiden mutation, homozygosity for the methylenetetrahydrofolate reductase gene (MTHFR) mutation, heterozygosity for the prothrombin gene mutation, homocysteinemia, and protein S deficiency, can cause miscarriage and serious complications of pregnancy.[1-10] The thrombophilic antiphospholipid syndrome (anticardiolipin antibodies [ACLAs] and/or the lupus anticoagulant) causes obstetric complications and recurrent miscarriage.[11-15] The documentation of thrombophilic causes of recurrent miscarriage is important.[1-15] Low–molecular weight heparin is effective in thromboprophylaxis of the thrombophilia of pregnancy.[12,13]

Plasminogen activator inhibitor (PAI-Fx) is the major inhibitor of fibrinolysis.[2,16,17] PAI-Fx is an independent and significant positive, potentially reversible (by metformin) risk factor for miscarriage in women with polycystic ovary syndrome (PCOS)[16] and in women with early recurrent miscarriage of unknown origin.[2,17] Gris et al[2,17] speculated that ''an impaired plasmin dependent proteolysis in women might favor recurrent abortion by promoting fibrin deposition in early placental circulation or by limiting trophoblast development, or both.'' Hypofibrinolysis is also associated with preeclampsia, intrauterine growth retardation (IUGR), abruptio placentae, and stillbirth.[18-23]

Our specific aim in the current study of 133 women with at least 1 pregnancy and measures of hypofibrinolytic and thrombophilic gene mutations was to determine retrospectively whether these mutations are associated with adverse pregnancy outcomes including prematurity, miscarriage, stillbirth, IUGR, eclampsia, and abruptio placentae.

SUBJECTS AND METHODS

Study Protocol

The study was performed with written informed consent, following a protocol approved by the Jewish Hospital Institutional Review Board. As part of the detailed coagulation measures, we previously performed polymerase chain reaction (PCR) assays for factor V Leiden, MTHFR, prothrombin, and PAI type 1 (PAI-1) gene mutations in studies of 204 women with atherothrombosis, osteonecrosis, and retinal vein thrombosis (102 with known gene mutations and 102 without known mutations). Our initial evaluation included a review of any known platelet abnormalities, including essential thrombocythemia. In most of these women, we also measured ACLAs immunoglobulin G (IgG) and IgM, as well as fasting serum homocysteine. Information was also gathered on race, age, and weight.

In the current study, we retrospectively obtained the history of pregnancy outcomes and clinically serious thrombotic events in the 204 women by questionnaire (Tables 1 and 2). To avoid duplication of our recent report that high PAI-Fx causes miscarriage in women with PCOS,[16] we excluded women with PCOS from the current study. There was no bias in the selection of these 204 women, who were identified in the temporal sequence of their referral to our center. Reproductive and pregnancy outcome histories were not known at the time the questionnaires were mailed.

To determine whether the PAI-1 genotype influences the response of PAI-Fx to treatment with metformin, we studied a separate group of 83 patients who were genotyped for the PAI gene mutation (51 4G/4G and 32 wild-type normal 5G/5G) and then subsequently treated with metformin 2.55 g/d for 3.6 and 3.1 months, respectively.[24,25]

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Submitted June 23, 1999; accepted January 4, 2000.

Supported by a grant from the Jewish Hospital Medical Research Council and the Jewish Hospital Lipoprotein Research Fund.

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0026-0495/00/4907-0015$10.00/0

doi:10.1053/mt.2000.6749

Table 1. Reproductive History Questionnaire

Item	Gene Mutation No.	Gene Mutation Mean ± SD	No Gene Mutation No.	No Gene Mutation Mean ± SD
Pregnant ≥1 time	72	(all white)	61	(2 black, 59 white)
Current age (yr)		57 ± 11		56 ± 9
Current weight (lb)		155 ± 36		165 ± 38
No. of pregnancies	221	3.07 ± 1.44	193	3.16 ± 1.51
		Mean ± SD per Subject (% of pregnancies)		Mean ± SD per Subject (% of pregnancies)
Full-term (≥37 weeks) live births	158	76 ± 31	149	80 ± 30
Live births <37 weeks (premature)	21	7 ± 17	7	5 ± 19
Miscarriages ≤13 weeks gestation	25	10 ± 20	24	9 ± 18
Fetal loss after 13 weeks	13	4.4 ± 13.6	6	2.3 ± 8.5
Stillbirths	2	0.7 ± 4.6	1	0.3 ± 2.6
Live births, fetal growth retardation (birth weight <5th percentile)	6	2.2 ± 12.7	0	
Physician-assisted terminations (elective abortions)	7	2.7 ± 9.4	7	3.0 ± 10.7
Pregnancies associated with eclampsia	11	4.4 ± 11.5	6	4.6 ± 16.3
Premature placental detachments	2	0.74 ± 4.55	1	0.33 ± 2.56
Never pregnant	13		14	
Ever try to become pregnant	1		3	
Medical condition preventing conception	1 (amenorrhea)		1 (endometriosis)	
Total	85		75	

Patients

Of 204 women with PCR assays for gene mutations, 160 (78%) answered the questionnaire and 133 of the 160 (83%) had at least 1 pregnancy. Of these 133 women, 126 (95%) had their PAI gene status determined; 122 of the 126 (97%) were genotyped for all 4 genes (factor V Leiden, prothrombin, MTHFR, and PAI-1; Table 3).

The 122 patients who were genotyped for all 4 genes were subdivided into 2 groups. The gene mutation group (n = 68) included those with 1 or more thrombophilic mutations (heterozygous for factor V Leiden, heterozygous for prothrombin, and homozygous for MTHFR) or hypofibrinolysis with homozygosity for the 4G polymorphism of the PAI-1 gene. The ''normal'' group (n = 54) included those with no mutation for all 4 genes (wild-type normal) or those with wild-type normal for the prothrombin and factor V Leiden mutations and heterozygosity for MTHFR and/or 4G/5G for the PAI-1 gene, neither heterozygosity associated with coagulation abnormalities (Table 3).

A separate analysis was performed for 126 women in whom the PAI-1 gene mutation was measured (Fig 1 and Table 3). A separate analysis was also performed for 96 women who were genotyped for all 4 genes, 42 with 4G/4G homozygosity and 54 with all 4 genes normal (Table 3).

Laboratory Methods

DNA and PCR methodology. Genomic DNA for each PCR assay was obtained by a salting-out procedure.[26] PCRs for the present study used primers and conditions as previously described.[9,27-31]

Measurement of ACLAs, homocysteine, and PAI-Fx. ACLAs IgG and IgM and homocysteine were analyzed using previously published methods.[30]

Pregnancy and Obstetrical History

The pregnancy and obstetrical history, along with the history of thrombosis were obtained retrospectively by a self-administered 25-item questionnaire (Tables 1 and 2). This form was subsequently reviewed with each patient by our medical staff. The diagnostic criteria for obstetric complications were from *Williams Obstetrics* (1997).[32] Previous studies have shown that self-administered pregnancy and obstetrical history questionnaires have an accuracy comparable to a review of hospital records and to guided external interviews.[33-39]

Retrospective information was obtained regarding type 1 and type 2 diabetes, gestational diabetes, and the diagnosis of alcoholism. Quantitative information was not obtained for maternal cigarette smoking or social alcohol intake during pregnancy. Information was not obtained for maternally restricted drug use (cocaine, morphine, and heroin) during pregnancy associated with an increased risk of miscarriage.[40] No questions were asked about pelvic inflammatory disease[41] or assisted reproduction,[42] both associated with a risk of first-trimester miscarriage.

Statistical Analysis

χ^2 analyses[43] were used to compare complications of pregnancy between women with and without thrombophilic and hypofibrinolytic gene mutations (Fig 1 and Table 3). Stepwise logistic regression[43] was used with complications of pregnancy as the dependent variable and age, 4G/4G status (5G/5G = 0, 4G/5G = 0, and 4G/4G = 1), MTHFR status (NN [normal] = 0, PN [polymorphism-heterozygosity] = 0, and PP [homozygosity] = 1), factor V Leiden status (NN = 0 and PN = 1), prothrombin gene status (NN = 0 and PN = 1), and number of pregnancies as explanatory variables. The stepwise regression model was also analyzed separately with ACLA IgG and IgM and homocysteine added to the explanatory variables.

To assess whether the 4 referral groups (atherothrombotic cardiovascular disease [n = 103], osteonecrosis of the jaw [n = 18], osteonecro-

Table 2. Major Thrombotic Events in Gene Mutation and Non–Gene Mutation Groups

Event	Gene Mutation (n = 85)	No–Gene Mutation (n = 75)
Phlebitis in leg veins (n)	8 (9%)	8 (11%)
Events	20	15
Occurring during pregnancy	3	2
Occurring on oral contraceptives, estrogen replacement	7	3
Pulmonary embolus (n)	2 (2%)	3 (4%)
Events	2	3
Occurring during pregnancy	0	1
Occurring on oral contraceptives, estrogen replacement	2	1
Osteonecrosis (n)	4 (5%)	14 (19%)
Events	11	34
Occurring during pregnancy	0	0
Occurring on oral contraceptives, estrogen replacement	2	4
Retinal vein thrombosis (n)	8 (9%)	5 (7%)
Events	14	5
Occurring during pregnancy	0	0
Occurring on oral contraceptives, estrogen replacement	8	1

Table 3. Pregnancy Complications Cross-Tabulated by Genotype

Group	Pregnancies	Live Births	Prematurity	First-Trimester Miscarriages	Late Miscarriages	IUGR	Eclampsia	Abruptio Placentae	Still Births	All Complications
Only consider PAI-1 gene (n = 126): significant variables in logistic regression, PAI-1 gene (+.057) and no. of pregnancies (+.0001)										
4G/4G (n = 43)	136	89	19	17	12	5	8	2	1	64
4G/5G (n = 55)	170	134	4	16	5	1	3	1	2	32
5G/5G (n = 28)	82	64	3	13	1	0	4	0	0	21
4G/4G *v* 4G/5G and 5G/5G		65% *v* 79%	14% *v* 3%	13% *v* 12%	9% *v* 2%	4% *v* 0.4%	7% *v* 3%			47% *v* 21%
		$\chi^2 = 7.9$,	$\chi^2 = 17.7$,	$\chi^2 = 0.08$,	$\chi^2 = 8.29$,	$\chi^2 = 6.2$,	$\chi^2 = 3.5$,			$\chi^2 = 28$,
		P = .005	*P* = .001	*P* = .77	*P* = .004	*P* = .012	*P* = .062			*P* = .001
All 4 genes tested (n = 122): significant variables in logistic regression, PAI-1 gene (+.029) and no. of pregnancies (+.0001)										
≥1 gene mutation (n = 68)	209	148	20	25	12	6	11	2	2	78
All 4 genes normal (n = 54)	170	133	6	19	5	0	4	1	1	36
		71% *v* 78%	10% *v* 4%	12% *v* 11%	6% *v* 3%	3% *v* 0%	6% *v* 3%			37% *v* 21%
		$\chi^2 = 2.7$,	$\chi^2 = 5.4$,	$\chi^2 = 0.06$,	$\chi^2 = 1.7$,	Fisher's	$\chi^2 = 2.2$,			$\chi^2 = 11.6$,
		P = .10	*P* = .021	*P* = .81	*P* = .19	*P* = .035	*P* = .14			*P* = .001
All 4 genes tested (n = 96): significant variables in logistic regression, no. of pregnancies (+.0001) and age (−.032)										
4G/4G (n = 42)	135	88	19	17	12	5	8	2	1	64
All 4 genes normal (n = 54)	170	133	6	19	5	0	4	1	1	36
		65% *v* 78%	14% *v* 4%	13% *v* 11%	9% *v* 3%	4% *v* 0%	7% *v* 3%			47% *v* 21%
		$\chi^2 = 6.42$,	$\chi^2 = 11.1$,	$\chi^2 = 0.1$,	$\chi^2 = 5.06$,	Fisher's	$\chi^2 = 3.2$,			$\chi^2 = 23$,
		P = .011	*P* = .001	*P* = .70	*P* = .025	*P* = .016	*P* = .07			*P* = .001

sis of the hip [n = 6], and retinal vein thrombosis [n = 6]) had significant self-selection bias, we compared between-group ACLA IgG and IgM and homocysteine (Newman-Keuls).[43] We used χ^2 analyses[43] to compare the distribution of the 4 genes between referral groups.

We also used χ^2 analyses to compare pregnancy outcomes for the women from the gene mutation group who had sustained a thrombotic event during pregnancy or estrogen therapy, versus those without thrombosis during pregnancy or estrogen therapy. Similarly, we compared pregnancy outcomes for women in the non–gene mutation group who sustained a thrombotic event during pregnancy or estrogen therapy versus those without thrombosis during pregnancy or estrogen therapy (Table 2).

PAI-Fx responses to metformin in 51 genotype 4G/4G subjects and 32 genotype 5G/5G subjects were evaluated by paired Wilcoxon test or

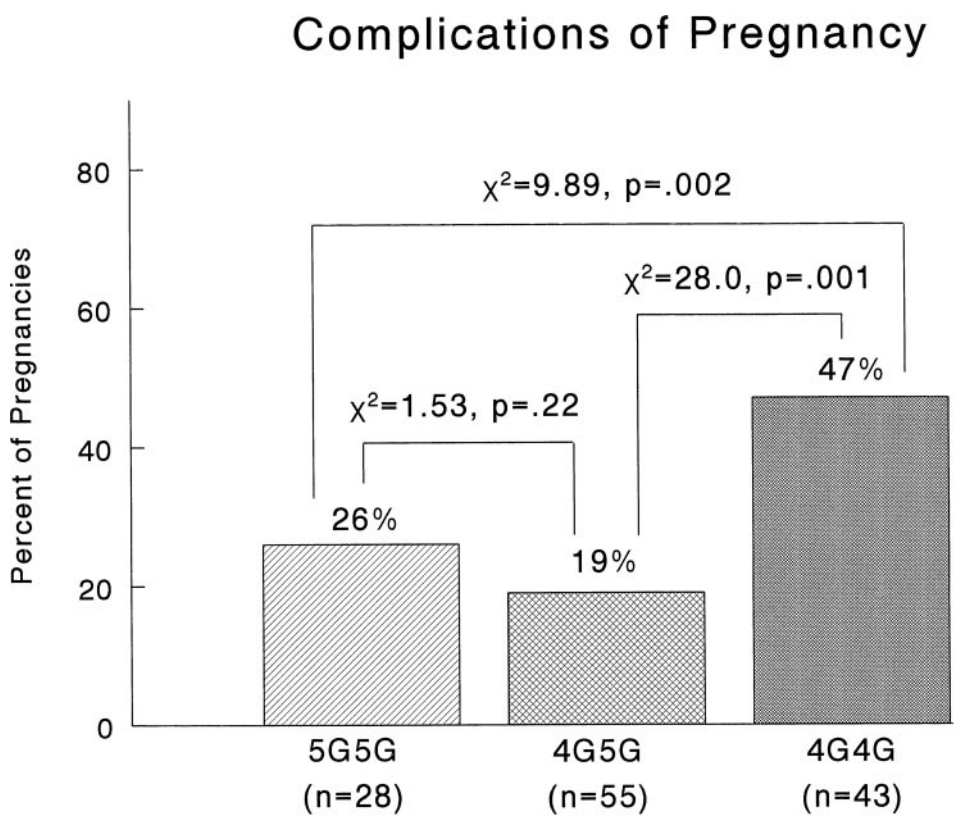

Fig 1. Complications of pregnancy in 126 women with determination of PAI-1 gene status: 28 wild-type normals (5G/5G), 55 heterozygotes for the 4G polymorphism (4G/5G), and 43 homozygotes (4G/4G).

paired *t* test according to their distribution.[43] The Wilcoxon test was used to compare treatment effects between the 2 groups.

RESULTS

Patient Characteristics

Of 102 letters sent to women with gene mutations, 85 (83%) were returned. Of the 85 women who replied, 72 (85%) had at least 1 pregnancy. Of these 72, 68 (94%) were genotyped for all 4 genes, and 43 (59%) were homozygous (4G/4G) for PAI-1 gene polymorphism (Table 4). Of 102 letters sent to those without gene mutations, 75 (74%) were returned. Of the 75 women, 61 (81%) had at least 1 pregnancy. Of these 61, 54 (89%) were genotyped for all 4 genes (Table 3). The distribution of the gene mutations did not differ in 44 women who failed to respond to the questionnaire versus 160 respondents ($P > .15$). At the time of their pregnancies, none of the patients had types 1 or 2 diabetes and none had alcoholism. None of the patients had a history of essential thrombocythemia.

Of 85 history respondents from the group of 102 women with gene mutations, 13 (15%) had never been pregnant, 12 by choice. One of these 13 patients had tried but failed to conceive. Of 75 history respondents from the group of 102 women without gene mutations, 14 (19%) had never been pregnant, 11 by choice; the other 3 had tried but failed to conceive (Table 1).

The cohort was predominantly caucasian. Pregnancy outcomes as a percentage of pregnancies (per subject) are shown in Table 1. Table 2 summarizes the major thrombotic events in the gene mutation and non–gene mutation groups.

The 133 women with 1 or more pregnancies had been referred to the Cholesterol Center for atherothrombotic cardiovascular disease (n = 103), osteonecrosis of the hip (n = 6), osteonecrosis of the jaw (n = 18), and retinal vein thrombosis (n = 6). To determine if these 4 referral groups had any self-selection bias, we compared ACLA IgG and IgM and homocysteine among the 4 groups. There were no group differences for these variables ($P > .1$). Similarly, we compared the distribution of factor V Leiden and prothrombin gene heterozygosity, 4G/5G polymorphism of the PAI-1 gene, and polymorphism of the MTHFR gene; there were no differences by referral group ($P > .1$). Moreover, there were no differences among the 4 referral groups for major complications of pregnancy ($P > .5$).

Pregnancy Outcomes Cross-Tabulated by Thrombophilic and Hypofibrinolytic Gene Mutations

Pregnancy outcomes did not differ ($P > .05$, data not shown) within gene mutation and non–gene mutation groups between women with a thrombotic event during pregnancy or estrogen therapy versus those without such events (Table 2).

Most of the 72 women in the gene mutation group had a single gene mutation; only 13 women had 2 gene mutations. Of 43 women who were homozygous for the 4G/4G polymorphism of the PAI-1 gene, 4 were also heterozygous for the Leiden mutation of the factor V gene, 8 were homozygous for the MTHFR mutation, and 1 was heterozygous for the prothrombin gene mutation (Table 4). Pregnancy outcomes did not differ in the 13 women with 2 gene mutations versus 59 women with 1 gene mutation ($P > .20$), excepting a higher first-trimester miscarriage rate (22% *v* 9%, $P = .03$).

Of 126 women with measurement of the PAI-1 gene, 43 were homozygous for the hypofibrinolytic 4G/4G polymorphism and 83 were either heterozygous (4G/5G, n = 55) or wild-type normal (5G/5G, n = 28). Complications of pregnancy did not differ ($P = .22$) between 4G/5G and 5G/5G subjects, but differed substantially between 4G/4G versus 5G/5G ($P = .002$) and 4G/5G ($P = .001$) (Fig 1 and Table 3).

Women with 4G/4G homozygosity (*v* 4G/5G and 5G/5G) had fewer live births (65% *v* 79%, $\chi^2 = 7.9$, $P = .005$), more prematurity (14% *v* 3%, $\chi^2 = 17.7$, $P = .001$), more second- and third-trimester fetal death (9% *v* 2%, $\chi^2 = 8.29$, $P = .004$), more intrauterine growth retardation (4% *v* 0.4%, $\chi^2 = 6.24$, $P = .012$), and more total complications of pregnancy (47% *v* 21%, $\chi^2 = 28.4$, $P = .001$). The number of pregnancies ($P = .0001$) and PAI-1 gene status ($P = .057$) were positively associated with complications of pregnancy by stepwise logistic regression when the age, number of pregnancies, and 4G/4G status were the explanatory variables (Table 3).

There were 122 women who were genotyped for all 4 genes, 68 with at least 1 gene mutation and 54 with all 4 genes normal. Of the 54 women with normal genes, all had wild-type normal factor V Leiden and prothrombin genes and 35 were 4G/5G and 19 5G/5G for the PAI-1 gene, 28 wild-type normal for MTHFR, 26 heterozygotes for MTHFR, and 12 wild-type normal for all 4 genes. Of 68 women with 1 or more gene mutations, there were 42 with 4G/4G homozygosity, 24 MTHFR homozygosity, 13 factor V Leiden heterozygosity, and 2 prothrombin gene heterozygosity. None of the 68 women with at least 1 gene

Table 4. Presence of One or More Thrombophilic or Hypofibrinolytic Mutations in 72 Patients (gene mutation group)

	Heterozygous for the Factor V Leiden Gene Mutation (n = 16)	Homozygous for the PAI-1 Gene Mutation 4G/4G (n = 43)	Homozygous for the MTHFR Gene Mutation (n = 24)	Heterozygous for the Prothrombin Gene Mutation (n = 2)
Heterozygous for the factor V Leiden gene mutation (n = 16)	12 had no other mutation	4	0	0
Homozygous for the PAI-1 gene mutation 4G/4G (n = 43)	4	30 had no other mutation	8	1
Homozygous for the MTHFR gene mutation (n = 24)	0	8	16 had no other mutation	0
Heterozygous for the prothrombin gene mutation (n = 2)	0	1	0	1 had no other mutation

mutation were homozygous for either the factor V Leiden or prothrombin gene mutations. Women with gene mutations (*v* normals) had more prematurity (10% *v* 4%, $\chi^2 = 5.4$, $P = .021$), more IUGR (3% *v* 0%, Fisher's $P = .035$), and more total complications of pregnancy (37% *v* 21%, $\chi^2 = 11.6$, $P = .001$). The number of pregnancies ($P = .0001$) and PAI gene 4G/4G status ($P = .029$) were positively associated with complications of pregnancy by stepwise logistic regression when the age, the number of pregnancies, and the 4 gene polymorphisms were explanatory variables (Table 3).

A subset of the 122 women with all 4 genes tested were separately evaluated to focus on 42 women with 4G/4G homozygosity, with little admixture by other gene mutations excepting 8 with homozygosity for the MTHFR polymorphism, 4 with heterozygosity for the factor V Leiden mutation, and 1 with heterozygosity for the prothrombin gene. Women with 4G/4G (*v* normals) had fewer live births (65% *v* 78%, $\chi^2 = 6.42$, $P = .011$), more prematurity (14% *v* 4%, $\chi^2 = 11.1$, $P = .001$), more second- and third-trimester fetal death (9% *v* 3%, $\chi^2 = 5.06$, $P = .025$), more IUGR (4% *v* 0%, Fisher's $P = .016$), and more total complications of pregnancy (47% *v* 21%, $\chi^2 = 23$, $P = .001$). The number of pregnancies was associated positively ($P = .0001$) and age inversely ($P = .032$) with complications of pregnancy by stepwise logistic regression when the age, number of pregnancies, and 4 gene mutations were the explanatory variables (Table 3).

Pregnancy Outcomes Cross-Tabulated by ACLA IgG and IgM and by Homocysteine

In 121 of 133 women, fasting serum homocysteine had been measured, and ACLA IgG and IgM in 100. There were no significant simple correlations ($P > .1$) between gene mutations for factor V Leiden, prothrombin, MTHFR, and PAI-1 and either absolute ACLA IgG and IgM and homocysteine or for these variables categorized as normal or high (>95th percentile for ACLA [IgG > 22 GPL, IgM > 10 MPL], >95th percentile [13.5 μmol/L] and >97.5th percentile [16.1] for homocysteine).

By χ^2 analysis, women with IgG greater than 22 GPL did not have more major complications of pregnancy than those with levels of 22 GPL or less ($\chi^2 = 0.37$, $P = .54$), and women with IgM greater than 10 MPL did not differ from those with IgM of 10 MPL or less ($\chi^2 = 1.5$, $P = .22$). Women with homocysteines greater than 13.5 μmol/L did not differ from those with a level of 13.5 or less ($\chi^2 = 0.33$, $P = .57$). However, women with homocysteine greater than 16.1 μmol/L had more major complications of pregnancy than those with levels of 16.1 or less (80% *v* 41%, $\chi^2 = 5.8$, $P = .016$).

The stepwise multiple logistic regression was reanalyzed with ACLA IgG and IgM and homocysteine added to the group of explanatory variables. ACLA IgG and IgM and homocysteine failed to enter the model as significant explanatory variables ($P > .05$). When ACLA IgG and IgM and homocysteine were added as categorical explanatory variables, subjects with homocysteine greater than 16.1 μmol/L had increased major complications of pregnancy ($P = .02$). There were no significant interaction terms of ACLA IgG or IgM or homocysteine with the gene mutations for major complications of pregnancy.

Reduction in PAI-Fx by Metformin in Patients With 4G/4G Versus 5G/5G Phenotype

Metformin therapy for a mean of 3.6 months decreased PAI-Fx from 22.5 ± 15.6 to 18.5 ± 11.3 U/L in 51 subjects with the 4G/4G genotype ($P = .067$). Metformin therapy for a mean of 3.1 months decreased PAI-Fx from 17.6 ± 11.9 to 14.2 ± 7.6 U/L ($P = .077$) in 32 subjects with the wild-type normal 5G/5G genotype. The percent decrement in PAI-Fx on metformin, 16% in the gene mutation group and 13% in the non–gene mutation group, did not differ by genotype ($P = .77$).

DISCUSSION

Clot lysis and modulation of the extracellular matrix is mediated by the plasminogen activation system.[44] The active fibrinolytic enzyme, plasmin, is derived from plasminogen, a circulating proenzyme. To facilitate cell migration within tissues and neovascularization, local dissolution of the basement membrane must be initiated by targeted proteolysis.[44] PAI-1 plays an important intravascular hemostatic role in arterial and venous thrombosis.[44-47] After acute arterial injury and thrombus formation, PAI-Fx is activated in endothelial cells and in smooth muscle.[44,45]

There is considerable controversy regarding whether the 4G/4G polymorphism of the PAI-1 gene is a risk factor for myocardial infarction or deep venous thrombosis.[47-68] PAI-Fx, the major inhibitor of fibrinolysis,[45-47] has been causally associated with coronary artery occlusion and predicts the reoccurrence of myocardial infarction in young men.[48-51] A common heritable 4G/5G single-nucleotide insertion or deletion polymorphism in the PAI-1 gene promoter region has been identified,[49] and is related to the circulating PAI-Fx level[50] and to hypofibrinolysis.[51] The prevalence of the 4G allele is significantly higher in patients with myocardial infarction before age 45 than in control subjects (allele frequency, 0.63 *v* 0.53).[49] In postmenopausal women with coronary artery disease, Grancha et al[62] reported that the 4G/5G polymorphism was related to coronary artery disease through increased PAI-Fx. In 500 Japanese subjects, Iwai et al[63] reported that ''the 4G/5G polymorphism of the PAI-1 gene influenced not only plasma PAI-1 antigen levels but also the time course of the progression to acute coronary syndromes in patients with coronary atherosclerosis.'' However, in the Physician's Health Study, Ridker et al[64] concluded that the 4G/5G polymorphism in the promoter of the PAI-1 gene is not a major pathogenic risk factor for arterial or venous thrombosis among middle-aged men. Similarly, in 200 survivors of myocardial infarction, Ardissino et al[65] found no association of the PAI mutation with the risk of myocardial infarction. In 331 men with a myocardial infarction and 302 controls. Doggen et al[66] concluded that the 4G/5G polymorphism of the PAI-1 gene was not associated with the risk of myocardial infarction.

Besides arterial occlusion,[48-50] high PAI-Fx has been identified as an independent risk factor for venous thrombosis and appears to be pathoetiologic for osteonecrosis, causing venous thrombosis in the head of the femur and in the jaws.[47,54-61] In 70 patients with deep venous thrombosis, Sartori et al[67] reported a positive association between venous thrombosis and 4G/4G polymorphism. However, in 158 patients with venous thrombo-

embolism, Stegnar et al[68] suggested that the 4G/5G mutation was not a major risk for venous thromboembolism.

In the current study, 68 women with at least 1 hypofibrinolytic and/or thrombophilic gene mutation were compared with 54 women with 4 normal or non–clinically significant mutations. Women with at least 1 gene mutation had more prematurity (10% *v* 4%, $P = .021$), more IUGR (3% *v* 0%, $P = .035$), and more total complications of pregnancy (37% *v* 21%, $P = .001$). The number of pregnancies ($P = .0001$) and 4G/4G homozygosity for the PAI-1 gene ($P = .029$) were positively associated with complications of pregnancy.

Of the women with testing for all 4 genes, 4G/4G homozygosity for the PAI-1 gene was the major determinant of complications of pregnancy, including fewer live births (65% *v* 78%, $P = .011$), more prematurity (14% *v* 4%, $P = .001$), more second- and third-trimester fetal death (9% *v* 3%, $P = .025$), more IUGR (4% *v* 0%, $P = .016$), and more total complications of pregnancy (47% *v* 21%, $P = .001$).

The common denominator for complications of pregnancy associated with thrombophilia[1-15] and hypofibrinolysis[2,16-23] is placental insufficiency. As the placenta rapidly establishes arteriovenous anastomoses with the endometrium and myometrium, excess thrombus formation produces placental insufficiency, which in turn causes miscarriage, eclampsia, stillbirth, IUGR, and abruptio placentae.[2,9,16,17] Early diagnosis of thrombophilic and/or hypofibrinolytic disorders in pregnancy is important, since low–molecular weight heparin therapy may ameliorate the complication of pregnancy in women with coagulation disorders.[12,13] The data in our current report are entirely congruent with those of our recent study of miscarriage in 41 women with PCOS,[16] where PAI-Fx was an independent risk factor for miscarriage.

Homozygosity for the 4G/4G polymorphism and the number of pregnancies were significant independent explanatory variables for serious complications of pregnancy. The hypofibrinolytic 4G/4G polymorphism appears to be causally associated with complications of pregnancy, probably by producing placental insufficiency through hypofibrinolysis.

In women attempting to conceive, it has been suggested that both miscarriage and nonpregnancy should be counted as failures, ''owing to the possibility of very early losses prior to the detection of pregnancy.''[69] Recurrent pregnancy loss (3 consecutive pregnancy losses prior to the 20th week of gestation) may be caused, in part, by anatomic disorders.[70] It has been estimated that up to 60% of women with anatomic disorders causing recurrent pregnancy loss can achieve successful pregnancy without any intervention.[70] Other causes of pregnancy loss include abnormal karyotypes.[71] Inner-city cocaine and tobacco use account for 24% of the risk of spontaneous abortion.[40]

In the present study, heritable hypofibrinolysis (4G/4G polymorphism of the PAI-1 gene) joins heritable thrombophilia[1-13] as a major independent risk factor for serious complications of pregnancy. Moreover, as in the current study, metformin decreases PAI-Fx to the same extent in patients who are homozygous for the 4G/4G polymorphism of the PAI-1 gene versus those with the 5G/5G genotype. By decreasing PAI-Fx,[72] metformin offers a potential avenue for reducing hypofibrinolysis-driven[2,16-20] complications of pregnancy.

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