

## MUTATIONS IN COAGULATION FACTORS IN WOMEN WITH UNEXPLAINED LATE FETAL LOSS

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**ABSTRACT**

**Background** The Factor V Leiden and prothrombin-gene mutations are independent risk factors for venous thrombosis; it is debated whether a mutation in the gene encoding methylenetetrahydrofolate reductase, an enzyme involved in homocysteine metabolism, also increases the risk of venous thrombosis. Whether any of these mutations is associated with an increased risk of late fetal death is not known.

**Methods** We studied 67 women with a first episode of unexplained late fetal loss (fetal death after 20 weeks or more of gestation) and 232 women who had had one or more normal pregnancies and no late fetal losses. All the women were tested for the presence of three gene mutations. Women with other thrombophilic conditions were excluded from the study.

**Results** Eleven of the 67 women with late fetal loss (16 percent) and 13 of the 232 control women (6 percent) had either the factor V or the prothrombin mutation. The relative risks of late fetal loss in carriers of the factor V and prothrombin mutations were 3.2 (95 percent confidence interval, 1.0 to 10.9) and 3.3 (95 percent confidence interval, 1.1 to 10.3), respectively. Thirteen percent of the women whose fetuses died and 20 percent of the control women were homozygous for the mutation in the methylenetetrahydrofolate reductase gene (relative risk, 0.8; 95 percent confidence interval, 0.5 to 1.2).

**Conclusions** Both the factor V and the prothrombin mutations are associated with an approximate tripling of the risk of late fetal loss. (N Engl J Med 2000;343:1015-8.)

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**I**N developed countries, approximately 1 in every 10 pregnancies ends in early death of the embryo or the fetus (that is, before 20 weeks of gestation), and 1 in every 200 pregnancies ends in late fetal loss.<sup>1-3</sup> Although fetal loss in the first trimester is a common complication of pregnancy and has many possible causes, fetal loss in the second and third trimesters is often associated with placental insufficiency.<sup>4</sup> Placental insufficiency resulting in fetal loss has been recognized in women with thrombophilic conditions clearly related to venous thromboembolism, such as the antiphospholipid-antibody syndrome<sup>5-8</sup> and deficiencies of antithrombin, protein C, and protein S.<sup>3,9,10</sup>

Recently, point mutations in the genes encoding coagulation factor V (a change from guanine to adenine at nucleotide 1691, referred to as Factor V Leiden) and prothrombin (a change from guanine to adenine at nucleotide 20210) have been found to be associated with thrombophilia.<sup>11,12</sup> Studies of the factor V mutation have revealed an association between the mutation and first- or second-trimester fetal loss.<sup>13-17</sup> Carriers of this mutation also seem to be at a higher risk for third-trimester fetal loss than non-carriers,<sup>3</sup> although this is not a consistent finding.<sup>10,16,17</sup> Whether the prothrombin-gene mutation is associated with fetal death is not known.<sup>3</sup> In addition, women who are homozygous for a cytosine-to-thymine mutation at nucleotide 677 in the gene encoding methylenetetrahydrofolate reductase (the primary methyl donor in the conversion of homocysteine to methionine), resulting in high plasma concentrations of homocysteine, are at higher risk for various obstetrical complications, such as preeclampsia, abruptio placentae, intrauterine growth retardation, and late fetal loss.<sup>18</sup> However, whether this mutation is associated with thrombophilia is still a matter of debate.<sup>19</sup>

We performed a case-control study in women with a first, unexplained late fetal loss to determine whether mutations in the genes coding for factor V, prothrombin, and methylenetetrahydrofolate reductase are associated with an increased risk of this complication of pregnancy. The demonstration of such an association has the potential to improve patient care through the use of anticoagulant drugs, which are effective in the prevention of complications of pregnancy in carriers of thrombophilia.<sup>20,21</sup>

**METHODS****Identification of Women with Late Fetal Loss and Controls**

Consecutive women 35 years old or younger who were referred between January 1995 and December 1998 to the two main obstetrical hospitals in Milan, Italy (which together are associated with more than 70 percent of births in the greater Milan area) because of late fetal loss (fetal death at 20 weeks or more of gestation)

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were eligible for the study. This gestational age was chosen according to the definition of late fetal death of the World Health Organization.<sup>1</sup> In order to avoid the inclusion of more severe cases, we excluded from the study women in whom thrombophilia was more likely, such as those who had had one or more previous late fetal losses; those with known independent risk factors for fetal death, such as uterine malformations and abnormal placental insertions; those who were known to abuse drugs or alcohol; those whose fetuses had abnormal karyotypes or congenital abnormalities; and those whose fetuses died from hydrops fetalis or erythroblastosis fetalis due to Rh incompatibility. Women were also excluded if they had had a multiple gestation with late loss of only one fetus. Control women were recruited during the same four-year period as the women whose fetuses died from among women living in the greater Milan area who gave birth to one or more healthy infants and had no history of late fetal loss. Nonwhite women were excluded from both groups, because the genetic mutations studied are rare in nonwhite populations.<sup>22,23</sup> Women were also excluded if they had a history of venous thrombosis or if they had any thrombophilic abnormalities, such as the antiphospholipid-antibody syndrome or deficiencies of antithrombin, protein C, or protein S. The study was approved by the institutional review boards of the participating hospitals, and all women gave written informed consent.

### Study Protocol

A complete history was obtained from all the women. The presence of the guanine-to-adenine mutation at nucleotide 1691 in the factor V gene, the guanine-to-adenine mutation at nucleotide 20210 in the prothrombin gene, and the cytosine-to-thymine mutation at nucleotide 677 in the methylenetetrahydrofolate reductase gene was determined by independent technicians according to previously described methods.<sup>24-26</sup> The tests were performed on peripheral-blood mononuclear cells from samples taken at least one month after late fetal loss. Serum antiphospholipid antibodies were measured as lupus anticoagulant (by tests including activated partial thromboplastin time, dilute Russell's-viper-venom time, kaolin clotting time, and silica clotting time assays) or anticardiolipin antibodies<sup>27</sup>; titers of more than 20 IgG or IgM phospholipid units were considered positive results. Plasma antithrombin, protein C, and protein S were measured as described elsewhere.<sup>28</sup> The placentas from the women whose fetuses died were examined by pathologists who were unaware of the presence or absence of mutations in the mother.

### Statistical Analysis

Relative risks and 95 percent confidence intervals were used as a measure of the association between late fetal death and each gene mutation. Among the women whose fetuses died, maternal age, week of gestation at the time of fetal death, weight of the fetus, and weight of the placenta were compared between those with and those without mutations of the genes for clotting factors by Student's *t*-test. The relative risk of late fetal loss associated with the presence of the mutations was adjusted for the effect of parity<sup>29</sup> by multiple logistic-regression analysis. Statistical analyses were performed with SAS software (version 6.12, SAS Institute, Cary, N.C.).

## RESULTS

Fifty-seven of 130 consecutive women with late fetal loss were excluded from the study. Fifteen of these women were nonwhite, 7 had more than one late fetal loss, 4 had uterine or placental malformations, 2 abused drugs, 12 had fetuses with congenital or genetic fetal abnormalities, 4 had fetuses with hydrops fetalis or erythroblastosis fetalis, 5 had multiple gestations that ended in the late loss of only one fetus, 7 had the antiphospholipid-antibody syndrome,

and 1 had protein C deficiency. Of the remaining 73 women, 67 (92 percent) agreed to participate in the study.

The characteristics of the women with late fetal loss and the control women are shown in Table 1. Of those whose fetuses died, 17 (25 percent) had had at least one successful previous pregnancy, whereas 10 (15 percent) had a history of fetal loss before 20 weeks of gestation. The frequency of hypertension was slightly higher among the control women, whereas that of diabetes mellitus and current smoking was similar in the two groups.

Overall, 11 of the 67 women with late fetal loss (16 percent) and 13 of the 232 control women (6 percent) had either the factor V or the prothrombin mutation (relative risk of late fetal loss, 3.3; 95 percent confidence interval, 1.4 to 7.8). All mutations were heterozygous, and none of the women carried both mutations. Among the women with late fetal loss, five (7 percent) had the factor V mutation and six (9 percent) had the prothrombin mutation, as compared with six (3 percent) and seven (3 percent), respectively, of the control women (Table 2). In the women with late fetal loss, the deaths occurred at a median maternal age of 31 years (range, 19 to 35) and a median gestational age of 26 weeks (range, 20 to 40). Fifty-one of the fetuses (76 percent) weighed less than 1500 g, nine (13 percent) weighed between 1500 and 2500 g, and seven (10 percent) weighed more than 2500 g. The maternal age at fetal loss, gestational week of fetal loss, and fetal weight were similar in the 11 women with and the 56 women without factor V or prothrombin mutations. The mean ( $\pm$ SD) weight of the placenta was also similar in the women with mutations and those without ( $315\pm 161$  and  $312\pm 191$  g, respectively).

Pathological examination was carried out in 62 of the 67 placentas from women with late fetal loss (93 percent). Fifteen of these placentas (24 percent) were normal, whereas in the remaining 47 (76 percent), intravascular thrombi, decidual vasculopathy, ischemic necrosis, or villous infarction was found. The placentas were abnormal in 7 (78 percent) of the 9 women with either factor V or prothrombin-gene mutations whose placentas were studied, as compared with 40 (75 percent) of the 53 women with no mutations. Intravascular thrombi were present in the placentas from 2 women with mutations and 23 without mutations, decidual vasculopathy in the placentas from 4 women with mutations and 9 without mutations, and villous infarction in the placentas from 1 woman with a mutation and 4 without mutations. Ischemic necrosis was seen in the placentas from four women with no mutations.

Nine women with late fetal loss (13 percent) and 46 control women (20 percent) were homozygous for the methylenetetrahydrofolate reductase gene mutation (Table 2). None of the nine women with late fe-

**TABLE 1.** CLINICAL CHARACTERISTICS OF THE WOMEN WITH LATE FETAL LOSS AND THE CONTROL WOMEN.

CHARACTERISTIC	WOMEN WITH LATE FETAL LOSS (N=67)*	WOMEN WITH NORMAL PREGNANCIES (N=232)
	no. (%)	
Primiparity	50 (75)	93 (40)
At least one successful pregnancy	17 (25)	232 (100)
History of fetal loss before week 20	10 (15)	39 (17)
Hypertension	3 (4)	29 (12)
Diabetes	2 (3)	1 (<1)
Current smoking	16 (24)	52 (22)

\*The women in this group had one pregnancy ending in fetal loss at 20 or more weeks of gestation.

tal loss were also carriers of either the factor V or prothrombin mutation. Of the 46 control women, 1 was also a carrier of the factor V mutation, and 2 were also carriers of the prothrombin mutation.

## DISCUSSION

This study demonstrates that women who are carriers of factor V or prothrombin mutations are at higher risk for late fetal loss than noncarriers. Placental thrombosis may be the underlying pathogenic mechanism of fetal death; thrombotic abnormalities were found in nearly 80 percent of the placentas of women in this cohort who were carriers of either mutation. Examination of the placentas also revealed thrombotic abnormalities in a similar proportion of women who had normal results on our screening for thrombophilic conditions. This finding suggests the presence of other, still unknown causes of placental thrombosis, although such thrombosis could be a nonspecific consequence of fetal loss. We did not find an association between the presence of homozygosity for the cytosine-to-thymine mutation of the methylenetetrahydrofolate reductase gene and late fetal loss.

We chose to investigate consecutive women with a first unexplained late fetal loss and to exclude from the study those with maternal or fetal conditions known to be associated with this event. Women more than 35 years of age were also excluded, because older women have an increased risk of adverse outcomes of pregnancy, including late fetal death.<sup>30-32</sup> Hence, our results are representative of young white women in Milan with a first episode of unexplained late fetal loss. Bias is unlikely to be present, for the following reasons. First, the two participating ob-

**TABLE 2.** PREVALENCE OF MUTATIONS IN THE GENES FOR FACTOR V, PROTHROMBIN, AND METHYLENETETRAHYDROFOLATE REDUCTASE IN WOMEN WITH LATE FETAL LOSS AND CONTROL WOMEN.

TYPE OF MUTATION	WOMEN WITH LATE FETAL LOSS (N=67)*	WOMEN WITH NORMAL PREGNANCIES (N=232)	RELATIVE RISK OF LATE FETAL LOSS (95% CI)†
	no. (%)		
Either factor V or prothrombin	11 (16)	13 (6)	3.3 (1.4-7.8)
Factor V	5 (7)	6 (3)	3.2 (1.0-10.9)
Prothrombin	6 (9)	7 (3)	3.3 (1.1-10.3)
Methylenetetrahydrofolate reductase	9 (13)	46 (20)	0.8 (0.5-1.2)

\*The women in this group had one pregnancy ending in fetal loss at 20 or more weeks of gestation.

†Relative risks have been adjusted for parity. CI denotes confidence interval.

stetrical hospitals serve as primary referral centers for greater Milan, covering more than 70 percent of all deliveries. Second, because all the women were white and lived in the same geographic area, variations in the frequency of gene mutations were minimized.<sup>22,23</sup> Third, interpretation bias was avoided by having the laboratory diagnosis made by technicians who were unaware of the characteristics of the study participants.

Our estimate of a tripling of the risk of late fetal loss in carriers of the factor V mutation is lower than the increase by a factor of approximately seven found in a previous case-control study.<sup>3</sup> This discrepancy can be explained by differences in study design. We studied consecutive women with late fetal loss but excluded women with recurrent losses, whereas the previous case-control study included a substantial proportion of women with more than one episode of late fetal loss (more than one third of all cases).<sup>3</sup> This, in combination with the relatively low prevalence of the factor V mutation among the control women in the previous study (2 percent),<sup>3</sup> might have led to the higher estimated risk of late fetal loss associated with the mutation. Although three other studies of family members with the factor V mutation found no increased risk of late fetal death, the 95 percent confidence intervals were wide, so a clinically important increase in risk cannot be excluded.<sup>10,16,17</sup>

What are the clinical implications of these results? Anticoagulant therapy is apparently effective in reducing the incidence of adverse pregnancy outcomes in women with another thrombophilic condition, the antiphospholipid-antibody syndrome.<sup>20</sup> This therapy could also favorably influence the outcome of pregnancy in women with thrombophilic mutations who

have had a pregnancy with late fetal loss. However, before considering anticoagulant therapy during subsequent pregnancies in these women, we need to know whether the presence of factor V or prothrombin mutations also predisposes women to recurrent unsuccessful pregnancies. Since nearly half the carriers of factor V or prothrombin mutations in our study had previously had at least one successful pregnancy, it cannot be assumed that these women are at risk for adverse outcomes of subsequent pregnancies. Moreover, long-term anticoagulant therapy is associated with a risk of bleeding and osteopenia.<sup>33</sup>

In conclusion, the probability of late fetal loss is three times as high among women who carry a factor V or prothrombin mutation as among women without these mutations. Since one or the other of these mutations was found in 16 percent of women with unexplained late fetal loss, screening for their presence in women with this complication of pregnancy is indicated. However, further studies of the risk-benefit ratio of anticoagulant treatment are needed before the therapy can be recommended for women with these mutations.

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