Mutant Maternal and Fetal Thrombophilic Genotypes as a Risk Factor for Preeclampsia

Lucia Maria Procopciuc[1], Gabriela Caracostea[2], Gabriela Zaharie[2], Mariana Puşcaş[2], Georgiana Iordache[2], Ileana Olteanu[1], F. Stamatian[2]

1. Department of Medical Biochemistry, "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca
2. Department of Gynecology, "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca

Correspondence:
Lucia Maria Procopciuc
e-mail: luciamariaprocopciuc@yahoo.com

Abstract

Objectives: *1. to determine the mutant thrombophilic genotypes (factor V Leiden mutation, 20210G→A mutation in the prothrombin gene, 677C→T and 1298A→C mutations in the metylenetetrahydropholate reductase gene) in the 26 pairs - preeclamptic mother/newborn of preeclamptic mother and 33 pairs - mother with normal pregnancy/newborn of mother with normal pregnancy 2. to calculate a risk score for different types of preeclampsia associated with the presence of genetic thrombophilias 3. to analyse the fetal thrombophilic genes in association with maternal genes as risk factors for preeclampsia.*

Methods: *Mutant and normal genotypes for the four thrombophilic mutations were determined using the PCR-RFLP methods. The relative risk (odds ratio- OR) and confidence interval (95%CI) were calculated. p values lower than 0.05 were considered statistically significant. The Fisher exact test table 2x2 was used.*

Results: *The risk of preeclampsia was 4.36 (p= 0.2), 5.55 (p = 0.003) and 1.25 (p= 0.8) in association with factor V Leiden, 677C→T and 1298A→C, respectively mutation. None of the subjects included the study was identified as positive for the 20210G→A mutation in the prothrombin gene. The risk of preeclampsia was significantly increased in the case of presence of multiple thrombophilias (OR 4.4, p = 0.019). The 677C→T mutation represents higher risk factor for mild and severe preeclampsia (OR 12.5, p = 0.03 and OR 19.6, p = 0.005, respectively). Multiple thrombophilias presence confers a risk of 7 (p = 0.02) for the severe forms of preeclampsia. The frequency of 677C→T mutation and multiple thrombophilia in newborns of preeclamptic mothers was significantly more increased compared to the frequency in the group of newborns from normal pregnant mothers (73.07% vs. 39.39% and 38.46% vs. 12.12%, respectively). The risk of preeclampsia is significantly increased when the 677C→T mutant genotypes or multiple thrombophilias are present both in the mother and the child (OR 6.53, p = 0.002 and OR 4.65, p = 0.06, respectively).*

Conclusions: *Our results emphasize the role of genetic thrombophilia in the development of different forms of preeclampsia. There is a mutant maternal/fetal genotype interaction, as a risk factor for preeclampsia. The presence of multiple thrombophilias in both the mother and the child is a risk factor for the development of preeclampsia.*

Keywords: *genetic thrombophilias, preeclampsia, PCR-RFLP methods*

Introduction

Pregnancy is a physiological state characterized by the expression of an increased thrombophilic potential as a result of increased procoagulant activity (the increased plasmatic level of certain coagulation factors) and decreased anticoagulation (reduced protein S, activated protein C - APC) or fibrinolysis[1,2,3]. Hypercoagulation is the result of the interaction between genetic risk factors and environmental factors.

Obstetric complications, repeated spontaneous abortion, preeclampsia, intrauterine fetal growth restriction, placental abruption, occur in 1-5% of pregnancies and are associated with maternal and fetal morbidity[4,5].

The etiology and pathogenesis of hypertensive disorders of pregnancy, including preeclampsia, pregnancy induced hypertension and HELLP syndrome, still remain incompletely known, but it is admitted that genetic predisposition, placental circulation abnormalities and hypercoagulation, maternal vascular endothelium abnormalities, thrombosis in the placental bed, contribute to placental ischemia[6,7].

The main thrombophilic risk factors are: factor V Leiden mutation (1691G→A), the 20210G→A mutation in the prothrombin gene, 677C→T and 1298A→C mutations in the methylenetetrahydropholate reductase gene (MTHFR)[8].

Factor V Leiden mutation, a point mutation through which the guanine from the 1691 nucleotide, exon 10, is substituted by adenine, is situated at one of the cleavage sites of the activated protein C, being associated with resistance to the degradation activity of the latter[9]. At the level of the mature protein, arginine is substituted by glutamine (Arg506Gln). The mutation has a frequency of 3-5% in the Caucasian population[10].

The 20210G→A mutation is also a point mutation by which the guanine from the 20210 nucleotide, 3' UTR region is substituted by adenine. The mutation is associated with increased plasma prothrombin levels (as a result of reduced mRNA stability), which involves an increased risk for thromboembolic events. This mutation has a prevalence of 1% in the general population[11].

The 677C→T mutation is a point mutation in the 677 nucleotide of the MTHFR gene, mutation which determines a decrease in the MTHFR activity, the major cause of hyperhomocysteinemia, a condition identified in 5-15% of the population[12,13]. The mutation determines the substitution of alanine with valine at position 222 (Ala222Val). Homozygotes for the T677 allele have an increased risk for deep venous thrombosis, pulmonary edema or preeclampsia. The frequency of this mutation in the Caucasian population is approximately 10%[14].

The 1298A→C mutation is a point mutation which consists of a substitution of the adenine from the 1298 nucleotide of the MTHFR gene with cytosine, also leading to a decrease in MTHRF activity. At the level of the mature protein, there is a substitution of glutamine with arginine (Glu429Ala)[15,16]. Double heterozygotes for C677T and A1298C have a 40 % activity of the enzyme and a biochemical profile similar to that given by the C677T mutation[17].

Severe preeclampsia is in many situations associated with preterm birth, being accompanied by numerous complications, both for the mother and the fetus. The risk of maternal mortality in the case of women who deliver preterm is increased 1.6-fold and extends to 2.71 in women with preeclampsia and preterm birth. Moreover, the risk of maternal mortality due to cardiovascular disorders is 8-fold increased in the case of women with preeclampsia and preterm birth, compared to women without preeclampsia and birth at term[18]. Unfortunately, the only solution for severe cases of preeclampsia is to perform a cesarean section.

Therefore, the determination of the exact etiological factors in preeclampsia, of the possible maternal and fetal risk factors, remains a priority for the obstetrician.

Study objectives:

1. To determine the mutant thrombophilic genotypes in the mother and fetus;

2. To calculate a risk score for preeclampsia associated with the presence of (single or multiple) genetic thrombophilias;

3. To analyze the distribution of the mutant thrombophilic genotypes, according to the degree of severity of preeclampsia;

4. To set down fetal mutant genes as independent risk factors for preeclampsia;

5. To analyze the fetal thrombophilic genes in association with maternal genes as risk factors for preeclampsia.

Materials and methods

Group selection

The studied groups were formed by 26 pairs - preeclamptic mothers/newborns of preeclamptic mothers and 33 pairs - mothers with normal pregnancy/newborns of mothers with normal pregnancy. The subjects enrolled in the study were recruited in the period September 2008 - September 2009. The patients diagnosed with preeclampsia were assigned to the moderate form of preeclampsia if their systolic blood pressure/diastolic blood pressure (SBP/DBP) was higher than 140/90mmHg, they were identified with increased blood pressure levels after the 20th week of gestation and they had urinary protein levels higher than 0.3g/24 hours. Preeclampsia was considered to be severe if the values of SBP/DBP were >160/100mmHg. The group of preeclamptic patients included 11 (44%) patients with pregnancy induced hypertension (PIH), 5 (20%) with moderate preeclampsia and 9 (36%) with severe preeclampsia. The control group included 33 patients with normal pregnancies. 18 (72%) preeclamptic women delivered at less than 37 weeks of gestation, compared to 4 (12.12%) women with normal pregnancies. There were no differences in the age of preeclamptic and normal pregnancy patients (years, 27.72 ± 4.74 vs 27.63 ± 4.95), but there were differences in SBP (mmHg, 156.4 ± 14.47 vs 126.52 ± 4.63) and DBP (mmHg, 105.2 ± 11.59 vs 76.3 ± 5.27), as well as in the antepartum body mass index (BMI) (kg/m2, 24.4 ± 4.5 vs 21.93 ± 3.07), maternal (92% vs 15.15%) and fetal complications (44% vs 0%), gestational age (weeks, 35.24 ± 3.88 vs 38.84 ± 1.09) and newborn birth weight (grams, 2491. 53 ± 1060.12 vs 3167.87 ± 413.73). In all cases p was less than 0.05.

The characteristics of patients with preeclampsia and of those with normal pregnancies are presented in Table 1.

The study was approved by the University Ethics Committee and the patients gave their written informed consent to participate in the study.

Methods

In order to determine the patients' genotypes. we drew 2ml of blood on EDTA from each patient (with or without preeclampsia) and 1ml of blood on EDTA from the fetus, postpartum. Mutant and normal genotypes for the four thrombophilic mutations were determined using the PCR-RFLP methods presented by Ridker, Poort, Frosst and van der Putt, with minor modifications[12,15,19,20]. The final amplification and enzymatic digestion conditions were the following:

1. Factor V Leiden mutation

PCR reaction: 20ng genomic DNA, 2.0mM $MgCl_2$, 200µM dNTP, 0.2µM primers, 0.625U Taq polymerase, Tannealing = 59° C.

Enzymatic digestion: 1X NE buffer 2, BSAX10, 2U restriction enzyme MnlI.

Fragments obtained: the normal G1691 allele formed three fragments of 104, 82 and 37bp, while the mutated A1691 allele formed two fragments of 141 and 82bp[19].

Table 1 Characteristics of the studied groups: preeclamptic pregnant women, women with normal pregnancies

	Preeclampsia (N=25)	Normal pregnant women (N= 33)	p
SBP, mean ± SD **DBP, mean ± SD**	**156.4 ± 14.47** **105.2 ± 11.59**	**126.52 ± 4.63** **76.3 ± 5.27**	<0.01 <0.01
PE type HTAIS, no. (%) Mild PE, no. (%) Severe PE, no. (%)	 11 (44%) 5 (20%) 9 (36%)	 - - -	
Pregnancy Monofetal, no. (%) Twin, no. (%)	 22 (88%) 3 (12%)	 32 (96.97%) 1 (3.03%)	
Age (years), mean ± DS <20 years, no. (%) 20-30 years, no. (%) 30-40 years, no. (%)	**27.72 ± 4.74** 2 (8%) 16 (64%) 7 (28%)	**27.63 ± 4.95** 3 (9.09%) 20 (60.6%) 10 (40%)	
Parity, mean ± DS Primiparous, no. (%) Secundiparous, no. (%) Three parous, no. (%) Multiparous, no. (%)	**1.36 ± 0.64** 18 (72%) 5 (20%) 2 (8%) -	**1.57 ± 1.09** 22 (66.66%) 7 (21.21%) 2 (6.06%) 2 (6.06%)	
Drug abuse Smoking, no. (%) Alcohol, no. (%) Oral contraceptives, no. (%)	 - - 3 (12%)	 1 (3.03%) - 4 (12.12%)	
BMI (kg/m^2), mean	**24.4 ± 4.5**	**21.93 ± 3.07**	<0.01
Maternal complications Changed renal function, no. (%) Changed hepatic function, no. (%) Oral glucose tolerance test, no. (%) Anemia, no. (%)	**23 (92%)** 3 (12%) 3 (12%) 3 (12%) 14 (56%)	**5 (15.15%)** 0 (0%) 0 (0%) 1 (0%) 4 (12.12%)	
Fetal complications Fetal hypotrophy, no. (%) Stillbirth, no. (%) Fetal distress, no. (%) Macrosomic fetus , no. (%) Prematurity, no. (%) Fetal cardiomyopathy, no. (%)	**11 (44%)** 4 (16%) 0 (0%) 4 (16%) 1 (4%) 1 (4%) 1 (4%)	**0 (0%)** 0 (0%) 0 (0%) 0 (0%) 0 (0%) 0 (0%) 0 (0%)	
Type of birth Spontaneous, no. (%) Cesarean, no. (%)	 4 (16%) 21 (84%)	 29 (87.87%) 4 (12.12%)	
Gestational age, weeks	35.24 ± 3.88	38.84 ± 1.09	<0.01
Newborn weight, average <1.500g, no. (%) 1.500-2.500g, no. (%) >2.500g, no. (%)	**2491.53 ± 1060.123** 3 (10.71%) 13 (46.43%) 12 (42.86%)	**3167.87 ± 413.73** 0 (0%) 3 (8.82%) 31 (91.18%)	<0.01

2. 20210G→A (prothrombin)

PCR reaction: 20ng genomic DNA 2.0mM $MgCl_2$, 200µM dNTP, 0.1µM primers, 0.625U Taq polymerase, Tannealing = 59°C.

Enzymatic digestion: 1X NE buffer 2, 5U restriction enzyme HindIII.

Fragments obtained: the normal G20210 allele formed one undigested fragment of 345bp, while the mutated A20210 allele formed two fragments of 322 and 23bp[20].

3. 677C→T (MTHFR)

PCR reaction: 20ng genomic DNA, 2.0mM $MgCl_2$, 200µM dNTP, 0.2µM primers, 0.625U Taq polymerase, Tannealing = 59°C.

Enzymatic digestion: 1X NE buffer 2, 5U restriction enzyme HinfI.

Fragments obtained: the normal C677 allele formed one undigested fragment of 198bp, while the mutated T677 allele formed two fragments of 175 and 23bp[12].

4. 1298A→C (MTHFR)

PCR reaction: 20ng genomic DNA, 2.0mM $MgCl_2$, 200µM dNTP, 0.2µM primers, 0.625U Taq polymerase, Tannealing = 62°C.

Enzymatic digestion: 1X NE buffer 2, 5U restriction enzyme MboII.

Fragments obtained: the normal A1281 allele formed 5 fragments of 56, 31, 30, 28 and 18 pb, while the mutated C1281 allele formed two fragments of 84, 31, 30, 18 pb[15].

All the reagents were from Fermentas, except for the primers which were from Eurogentec.

Statistical analysis: in order to compare the values of SBP, DBP, age, parity, BMI, gestational age, and newborn weight in the two groups, we used the Student test. The relative risk (odds ratio- OR) and confidence interval (95%CI) were calculated. p values lower than 0.05 were considered statistically significant. The Fisher exact test table 2x2 was used.

Results

None of the sujects included in the study was identified as positive for the 20210G→A mutation in the prothrombin gene. Of the 25 pregnant women with preeclampsia, 19 (76%) presented at least one of the three thrombophilic mutations (factor V Leiden, C677T, A1298C), compared to 17 of the 33 (51.51%) pregnant women with normal pregnancy courses (OR 2.98, 95%IC [0.95- 9.36], p = 0.05). The frequency of the three mutations identified was higher in the preeclampsia group compared to the control group. Thus, the factor V Leiden mutation was identified in 12% of patients, compared to 3.03% in the control group. The risk of preeclampsia was 4.36 (95%CI [0.43- 44.73], p = 0.2) in association with this genetic variation. All the positive subjects were heterozygous. The 677C→T mutation was identified in 64% of patients compared to 24.24% in the control group. The risk of preeclampsia in association with this mutation was of 5.55, (95%CI [1.78-17.38], p = 0.003). The 677C→T mutation had the following genotype distribution: CT- 10 (40%) vs 6 (18.18%), TT- 6 (24%) vs 2 (6.06%). The last mutation identified in the two groups was 1298A→C, 48% in preeclamptic patients and 42.42% in the control group. The risk of preeclampsia in patients positive for this genetic variation was 1.25 (95%CI [0.44- 3.56], p = 0.8). This mutation had the following genotype distribution: AC- 10 (40%) vs 12 (36.36%), CC- 2 (8%) vs 2 (6.06%). The thrombophilic mutation distribution in the two groups (patients with preeclampsia vs. patients with normal pregnancies) and the risk of preeclampsia in association with the hetero- and homozygous genotypes are presented in Table 2.

Table 2 Thrombophilic mutations in the two study groups: women with preeclampsia and control women

Genotype	Preeclampsia (N= 25)	Control (N = 33)	OR, 95%IC	p
Factor V Leiden, no. (%)	**3 (12%)**	**1 (3.03%)**	4.36 [0.43-44.73]	0.2
GG1691 genotype, no. (%)	22 (88%)	32 (96.96%)		
AG1691 genotype, no. (%)	3 (12%)	1 (3.03%)	4.36 [0.43-44.73]	0.2
AA1691 genotype, no. (%)	0 (0%)	0 (0%)		
A1691 allele, no. (%)	3 (0.06)	1 (0.015)	4.14 [0.42-41.14]	0.2
677C→T, no. (%)	**16 (64%)**	**8 (24.24%)**	5.55 [1.78-17.38]	**0.003**
CC677 genotype, no. (%)	9 (36%)	25 (75.75%)		
CT677 genotype, no. (%)	10 (40%)	6 (18.18%)	3 [0.91-9.89]	0.3
TT677 genotype, no. (%)	6 (24%)	2 (6.06%)	4.89 [0.89-26.78]	**0.05**
T677 allele, no. (%)	22 (0.44)	10 (0.15)	4.4 [1.84-10.55]	**<0.01**
1298A→C, no. (%)	**12 (48%)**	**14 (42.42%)**	1.24 [0.44-3.56]	0.7
AA1298 genotype, no. (%)	13 (52%)	19 (57.57%)		
AC1298 genotype, no. (%)	10 (40%)	12 (36.36%)	1.17 [0.4-3.39]	0.7
CC1298 genotype, no. (%)	2 (8%)	2 (6.06%)	1.35 [0.17-10.29]	0.5
C1298 allele, no. (%)	14 (0.28)	16 (0.24)	1.22 [0.53-2.8]	0.6
Single thrombophilic defect, no. (%)	8 (32%)	12 (36.36%)	0.82 [0.27-2.47]	0.47
Multiple thrombophilias, no. (%)	11 (44%)	5 (15.15%)	4.4 [1.28-15.15]	**0.019**

Our study also aimed to establish a correlation between the presence of mutant thrombophilic genotypes and the severity of preeclampsia. Thus, factor V Leiden mutation was identified with a frequency of 9.09%, 20% and 11.11%, respectively, in the subgroups of patients with PIH, mild and severe preeclampsia, compared to a percentage of only 3.03 in the control group. The 677C→T mutation was also identified with an increased frequency in the mild (80%) and severe (77.77%) preeclampsia subgroups, compared to 24.24% in the control group. In the PIH subgroup, the frequency of this polymorphism was 45.45%. The 1298A→C mutation was identified with a frequency of 55.55% and 54.55% in the severe preeclampsia and PIH subgroups, respectively, compared to 42.42% in the control group. The frequency of this polymorphism was 20% in the mild preeclampsia subgroup. The distribution of the three polymorphisms and the risk of pregnancy induced hypertension, mild and severe preeclampsia are presented in Table 3.

Pregnant women with or without preeclampsia were divided into two groups: patients who delivered at more than 37 weeks of gestation and at less than 37 weeks of gestation. Differential analysis according to gestational age at birth suggests that the percentage of preeclamptic women with mutant thrombophilic genotypes who delivered at less than 37 weeks of gestation (13/25- 30.43%) was higher than the percentage of preeclamptic women with mutant thrombophilic genotypes who delivered at more than 37 weeks of gestation (6/25- 13.04%) (OR 3.43, 95%CI [1.02-11.48], p = 0.03).

The comparative analysis of the two newborn groups, from preeclamptic and normal pregnant mothers, shows

Table 3 Degree of severity of genetic thrombophilia and preeclampsia

Genetic thrombophilia	HTAIS (N = 11)	Mild preeclampsia (N = 5)	Severe preeclampsia (N = 9)	Normal pregnancies (N = 33)
Factor V Leiden **no. (%)** **OR, 95%IC** **p**	1 (9.09%) 3.2 [0.18-55.95] 0.4	1 (20%) 8 [0.41-154.43] 0.2	1 (11.11%) 4.57 [0.25-82.25] 0.3	1 (3.03%)
677C→T, **no. (%)** **OR, 95%IC** **p**	5 (45.45%) 2.6 [0.62-10.87] 0.25	4 (80%) 12.5 [1.21-128.66] **0.03**	7 (77.77%) 19.6 [3.12-123.9] **0.005**	8 (24.24%)
1298A→C, **no. (%)** **OR, 95%IC** **p**	6 (54.55%) 1.63 [0.41-6.43] 0.5	1 (20%) 0.34 [0.03-3.37] 0.6	5 (55.55%) 2.26 [0.46-11.08] 0.7	14 (42.42%)
Single thrombophilic defect, **no. (%)** **OR, 95%IC** **p**	5 (45.45%) 1.45 [0.37-5.81] 0.4	2 (40%) 1.17 [0.17-7.99] 0.6	2 (22.22%) 0.58 [0.1-3.35] 0.6	12 (36.36%)
Multiple thrombophilias, **no. (%)** **OR, 95%IC** **p**	4 (36.36%) 3.2 [0.68-15.14] 0.19	2 (40%) 3.06 [0.4-23.4] 0.2	5 (55.55%) 7 [1.38-35.48] **0.02**	5 (15.15%)

Table 4 Distribution of genetic thrombophilia in the groups of newborns of preeclamptic mothers and normal pregnant mothers

Thrombophilic mutations	Newborns of preeclamptic mothers (N = 26)	Newborns of normal pregnant mothers (N= 33)	OR, 95%IC	p
Factor V Leiden, no. (%)	1 (3.84%)	1 (3.03%)	1.28 [0.07-21.49]	0.6
677C→T - MTHFR, no. (%)	19 (73.07%)	13 (39.39%)	4.17 [1.37-12.7]	**0.017**
1298A→C-MTHFR, no. (%)	12 (46.15%)	8 (24.24%)	2.67 [0.88-8.11]	0.06
Single defect, no. (%)	12 (46.15%)	14 (42.42%)	1.16 [0.41-3.27]	0.7
Multiple thrombophilias, no. (%)	10 (38.46%)	4 (12.12%)	4.53 [1.22-16.8]	**0.03**

Table 5 Interaction between mutant maternal and fetal thrombophilic genotypes as a risk factor in preeclampsia

	Factor V Leiden	677C→T	1298A→C	Multiple thrombophilias
Newborns +/mothers - PE **Newborns +/mothers - control** **OR, 95%IC** **p**	1/26 (3.84%) 1/33 (3.03%) 1.28 [0.07-21.49] 0.6	6/26 (23.08%) 8/33 (24.24%) 0.93 [0.28-3.15] 0.5	5/26 (19.23%) 0/33 (0%)	6/26 (24%) 2/33 (6.06%) 4.65 [0.85-25.36] 0.06
Newborns +/mothers + PE **Newborns+/mothers - control** **OR, 95%IC** **p**	0/26 (0%) 0/33 (0%)	14/26 (53.85%) 5/33 (15.15%) 6.53 [1.91-22.23] **0.002**	8/26 (30.77%) 8/33 (24.24%) 1.39 [0.44-4.39] 0.39	6/26 (24%) 2/33 (6.06%) 4.65 [0.85-25.35] 0.06
Newborns -/mothers +PE **Newborns-/mothers + control** **OR, 95%IC** **p**	3/26 (11.54%) 1/33 (3.03%) [0.4-42.72] 0.3	2/26 (7.69%) 3/33 (9.09%) 0.83 [0.13-5.4] 0.6	4/26 (15.38%) 6/33 (18.18%) 0.81 [0.2-3.26] 1	4/26 (15.38%) 3/33 (9.09%) 1.81 [0.37-8.96] 0.6
Newborns -/mothers - PE **Newborns -/mothers - control** **OR, 95%IC** **p**	22/26 (84.62%) 31/33 (93.93%) 0.35 [0.06-2.11] 0.3	4/26 (15.38%) 17/33 (51.51%) 0.17 [0.04-0.6] 0.005	9/26 (34.61%) 19/33 (57.57%) 0.39 [0.13-1.1] 0.1	12/26 (46.15%) 26/33 (78.78%) 0.23 [0.07-0.72] 0.01

the following distribution of polymorphisms: factor V Leiden - 1 (3.84%) vs 1 (3.03%), 677C→T- 19 (73.07%) vs 13 (39.39%), 1298A→C- 12 (46.15%) vs 8 (24.24%). The distribution of the thrombophilic mutations in the two groups of newborns is shown in table 4.

The interaction between mutant maternal and fetal thrombophilic genotypes are presented in table 5.

Discussion

The presence of thrombophilic mutations associated with a hypercoagulability status leads to endothelial lesions, abnormal fibrin deposits and the occurrence of microthrombi in the maternal blood flow[7,21]. Among the thrombophilic factors potentially involved in the development of preeclampsia, we mention factor V Leiden mutation, the 20210G→A mutation in the prothrombin gene, the homozygous status for the 677C→T mutation in the methylenetetrahydrofolate reductase gene (MTHFR). Results regarding the involvement of genetic thrombophilia in preeclampsia are controversial. There are studies which confirm the involvement of factor V Leiden mutation, the 20210G→A mutation in the prothrombin gene, 677C→T in the MTHFR gene or multiple thrombophilias in the development of preeclampsia[22-26].

A study designed by Makatsariya (2009) shows that the essential pathogenic mechanism in preeclampsia is represented by thrombophilia. Moreover, it suggests that preconception low molecular weight heparin, antioxidant and vitamin treatment prevents the moderate or severe forms of preeclampsia in a percentage of 100. Preeclamptic patients require anticoagulant therapy during their future pregnancies[27]. Studies only including white women have an increased frequency for factor V Leiden mutation and the 20210G→A mutation in the prothrombin gene compared to those including black or Hispanic women[28]. A study conducted by Driul (2005) in 103 preeclamptic patients shows a frequency of 17.4% for the factor V Leiden mutation and 1.5% for the 20210G→A mutation, respectively. Also, the frequency of TT677 homozygotes is 21.7% in the preeclamptic group[29]. Another study conducted by Best in 2009 shows that there is no association between factor V Leiden and 20210G→A mutation in an Indian population[30]. Two studies performed by Morrison (2002) in 110 women with preeclampsia and also by Livingstone (2001) have shown that factor V Leiden, 20210G→A and 677C→T fetal and maternal genes do not represent risk factors for severe preeclampsia, HELLP syndrome or pregnancy induced hypertension[22,31]. Hauge, in a study which targeted severe preeclampsia patients reveals an increased 1298A→C mutation frequency in the MTHFR gene[32]. Other studies do not confirm this association[33,34]. Also, the presence of more than one thrombophilic defect substantially increases the risk of obstetric complications[35].

In our study, thrombophilic mutations were more frequent in the preeclamptic group compared to the control group. The heterozygous genotype (GA) for the factor V Leiden mutation was associated with a risk factor of 4.36 (p = 0.2) for the occurrence of preeclampsia. The heterozygous (CT) and the homozygous (TT) genotype for the 677C→T mutation was associated with a risk factor for the occurrence of preeclampsia of 3 (p = 0.06) and 4.89 (p = 0.05), respectively. In the case of the 1298A→C mutation, the heterozygous (AC) and the homozygous (CC) genotypes were associated with a risk of 1.17 (p = 0.7) and 1.35 ((p = 0.5) for the development of preeclampsia, respectively. The risk of preeclampsia was higher in the presence of two thrombophilic mutations and three thrombophilic mutations, respectively, compared to that of only one mutant genotype present. The presence of a single thrombophilic factor did not represent a risk for the

development of preeclampsia, at least in this study group (32% vs 36.6%, OR 0.82, 95%CI [0.27-2.47], p = 0.47). The risk was significantly increased in the case of the presence of multiple thrombophilias (44% vs 15.15%, OR 4.4, 95%CI [1.28-15.15], p = 0.019) (Table 2).

An Italian study conducted by Mello in 2005, which analyzed the frequency of thrombophilic mutations according to the degree of severity of preeclampsia, found an increased frequency of genetic thrombophilia (50.7%) compared to the frequency found in the control group (17.2%), with a 4.9 risk factor for preeclampsia. In contrast, no association was found between genetic thrombophilia and the moderate form of preeclampsia[28]. In our study, the analysis of genetic thrombophilia according to the degree of severity of preeclampsia suggests that factor V Leiden mutation confers a a risk of 8 (p = 0.2) for mild preeclampsia and 4.57 (p = 0.3) for the severe type. The risk of development of pregnancy induced hypertension is 2.6 (p = 0.25) when the 677C→T mutation is present. The risk increases to 12.5 (p = 0.03) and 19.6 (p = 0.005) for mild and severe forms of preeclampsia, respectively. The 1298A→C mutation represents a risk factor for pregnancy induced hypertension (OR 1.63, p = 0.50) and severe preeclampsia, respectively (OR 2.26, p= 0.7). The presence of a single thrombophilic factor confers a moderate risk for mild preeclampsia (OR 1.17, p = 0.6), but does not represent a risk for the severe form (OR 0.58, p = 0.6). The risk increases in the case of the presence of multiple thrombophilias, which confers a risk of 3.2 (p = 0.19) and 3.06 (p = 0.2) for the HTAIS and mild preeclampsia, respectively. The risk increases to 7 (p= 0.02) for the severe forms of preeclampsia (Table 3).

Results are also controversial regarding the severity of preeclampsia and gestational age at birth. Most studies have found an association between severe preeclampsia and gestational age lower than 34 weeks and genetic thrombophilia, association which has not been identified for mild preeclampsia or pregnanacy induced hypertension[36]. Regarding gestational age and newborn birth weight, no significant differences have been found between preeclamptic women with genetic thrombophilia and preeclamptic women without genetic thrombophilia. However, we noted that of the 16 pregnant women who delivered at less than 37 weeks and who were positive for at least one of the studied thrombophilic variations, 13 (81.25%) had preeclampsia compared to the 5 of 19 (26.32%) thrombophilic pregnant women who delivered at more than 37 weeks and had preeclampsia (OR 12.13, 95%CI [2.4-61.2], p = 0.002).

Fetal thrombophilic genes and the risk of preeclampsia

Designing a genetic profile for preeclampsia involves, as potential genetic mechanisms, genetic maternal-fetal interactions.

There is a hemodynamic balance between fetal and maternal circulation, with a determining role in the evolution of pregnancy and the normal development of the fetus. It has been speculated that fetal thrombophilic mutations destroy this equilibrium, leading to the occurrence of placental microthrombosis which influences fetoplacental blood flow, thus contributing to the pathogenesis of preeclampsia. One explanation would be that the maladaptation of the immune system, including endothelial cell activation, placental leucocyte and monocyte activation, contributes to placental ischemia and vessel anomalies which determine preeclampsia[37]. Some studies have attempted to establish the role of mutant factor V Leiden, 20210G→A and MTHFR genes in the occurrence of obstetric complications, including preeclampsia[38,39]. Schlembach studied the mutant mother/child genotype association which plays a role in intrauterine growth restriction[37]. Another study performed by Ariel in 2004 showed that neither maternal nor fetal thrombophilia represents a risk factor for vascular lesions of the placenta[40].

In order to analyze the involvement of fetal genes in the development of preeclampsia in the mother, we analyzed in our study two groups of newborns of preeclamptic (N = 26) and normal pregnant (N = 33) mothers, and also, we conducted a comparative analysis of the mutant mother/child genotype interaction for the three genetic variations and the risk of preeclampsia. Thrombophilic mutations were identified with a higher frequency in the group of newborns of preeclamptic mothers, compared to the group of newborns of mothers with normal pregnancies (Table 4).

The frequency of factor V Leiden (OR 1.28, 95%CI [0.07-21.49], p = 0.6), 677C→T (OR 4.17, 95%CI [1.37-12.7], p = 0.017) and 1298A→C (OR 2.67, 95%CI [0.88- 8.11], p = 0.06) mutations in newborns of preeclamptic mothers was more increased compared to the frequency in the group of newborns from normal pregnant mothers (Table 4). It is also interesting that multiple thrombophilia in newborns of preeclamptic mothers was significantly higher compared to the group of newborns of normal pregnant mothers (38.46% vs 12.12%, OR 4.53, 95%CI [1.22- 16.8], p = 0.03).

The presence of factor V Leiden mutation in the newborn in the absence of the mutant genotype in the mother determines a risk of 1.28 (p = 0.6) for the development of preeclampsia. At the same time, the presence of this mutation in the mother and its absence in the child confers a risk factor of 4.17 (p = 0.3). Regarding the 677C→T mutation, we found that the mutant genotype in the newborn in its absence in the mother does not represent a risk factor for preeclampsia (OR 0.9, p = 0.5). The presence of the mutant genotype in the mother and its absence in the child is not a risk factor for preeclampsia either (OR 0.8, p = 0.6). The risk is however significantly increased when the mutant genotypes are present both in the mother and the child (OR 6.53, p = 0.002). The simultaneous presence of the 1298A→C mutation in the mother and child is associated with a risk of 1.39 (p = 0.39) for the development of preeclampsia. The mutant 1298A→C genotype in the mother alone does not induce preeclampsia. Mutiple thrombophilias only in the mother represents a moderate risk for the occurrence of preeclampsia (OR 1.81, p = 0.6, respectively). However, the risk increases when multiple thrombophilias are present only in the child or in both the

Acknowledgement:
The results of this study were obtained with the financial support of the research grant IDEI number 1210/2008 (project cod 1338/2008), with the title: "Genetic model involved in increased perinatal maternal-fetal morbidity in patients with preeclampsia. A new theory for diagnosis, treatment and prophylaxis of preeclampsia"
The results published in the paper "Influence of RAS polymorphisms on the development and perinatal outcome of preeclampsia. Genetic RAS evaluation" (authors Lucia Procopciuc, Gabriela Caracostea, Georgiana Iordache, Ileana Olteanu, F. Stamatian) in the journal "Gineco.ro - A Journal of Obstetrics and Gynecology", vol. 5, no. 2, 2009 were obtained with the financial support of the research grant IDEI number 1210/2008 (project cod 1338/2008) with the title: "Genetic model involved in increased perinatal maternal- fetal morbidity in patients with preeclampsia. A new theory for diagnosis, treatment and prophylaxis of preeclampsia"

child and the mother (OR 4.65, p = 0.06 and OR 5.71, p = 0.06, respectively) (Table 5).

Conclusions

In conclusion, our results emphasize the role of genetic thrombophilia in the development of different forms of preeclampsia. Our study also shows the possible involvement of fetal thrombophilic genes in the occurrence of preeclampsia, but does not confirm the hypothesis according to which fetal thrombophilic mutations may cause preeclampsia independently of the maternal genotype. There is a mutant maternal/fetal genotype interaction, as a risk factor for preeclampsia. The presence of combined thrombophilias in both the mother and the child is a risk factor for the development of preeclampsia.

In order to confirm these results, further studies regarding this association are needed, by the investigation of a greater number of mother/child pairs. ■

References

1. Comp P.C., Thurnau G.R., Welsh J., et al. Functional and immunologic protein S levels are decreased during pregnancy. Blood 1986; 68: 881-885.
2. Cumming A.M., Tait R.C., Fildes S. Development of resistance to activated protein C during pregnancy. Br J Haematol 1995; 90: 725-727.
3. Stirling Y., Woolf L., North W.R.S. Haemostasis in normal pregnancy. Thromb Haemost 1984; 52: 176-182.
4. Roberts J.M., Taylor R.N., Musci T.J., et al. Preeclampsia: an endothelial cell disorder. Am J Obstet Gynecol 1989; 161: 1200-1204.
5. Salafia C.M., Pezzulo J.C., Lopez-Zeno J.A., et al. Placental pathologic features of preterm preeclampsia. Am J Obstet Gynecol 1995; 173: 109- 1105.
6. Kupferminc M.J., Fait G., Many A., et al. Severe preeclampsia and high frequency of genetic thrombophilic mutations. Obstet Gynecol 2000; 96: 45-49.
7. Dekker G.A., Sibai B.M. Etiology and pathogenesis of preeclampsia: current concepts. Am J Obstet Gynecol 1998; 179: 1359-1375.
8. Roberts J.M., Cooper D.W. Pathogenesis and genetics of preeclampsia. Lancet 2001; 357: 53-56.
9. Bertina R.M., Koeleman B.P.C., Koster T., et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369: 64-67.
10. Rees D.C., Cox M., Clegg J.B. World distribution of factor V Leiden. Lancet 1995; 346: 1133-1134.
11. Poort S.R., Rosendaal F.R., Reitsma P.H., et al. A common genetic variation in the 3'untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996; 88: 3698- 3703.
12. Frosst P., Blom H.J., Milos R. et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995; 10: 111-113.
13. Brattstrom L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. Circulation 1998; 98: 2520-2526.
14. Botto L.D.. Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am. J. Epidemiol. 2000; 151: 862-877.
15. van der Put N.M., Steegers-Theunissen R.P., Frosst P. et al. Mutated methylenetetrahydrofolate reductase as a risk factor for sfina bifida. Lancet 1995; 346: 1071-1071.
16. Yamada K., Chen Z., Rozen R., et al. „Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase". Proc. Natl. Acad. Sci. U.S.A. 2001; 98 (26): 14853-8.
17. Wisberg I., Tran P., Christensen B., et al. A second genetic polymorphism in the methylene tetrahydropholate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 1998; 64: 169-172.
18. Irgens H., Reisaeter L., Irgens L.M., et al. Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. British Medical Journal, 2001.
19. Ridker P.M., Hennekens C.H., Lindpaintner K. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke and venous thrombosis in apparently healthy men. N Engl J Med 1995; 332:912- 917.
20. Poor S.R., Rosendaal F.R., Reitsma P.H., et al. A common genetic variation in the 3' untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venosu thrombosis. Blood 1996; 88: 3698- 703.
21. Kupferminc M., Eldor A. Stainman N., et al. Increased frequency of genetic thrombophilia in women with complications of pregancy. N Engl J Med 1999; 341(5): 384.
22. Morrison E.R., Miedzybrodzka Z.H., Campbell D.M. Prothrombotic genotypes are not associated with pre-eclampsia and gestational hypertension: results from a large population-based study and systematic review. Thromb Haemostasis 2001; 87: 779-785.
23. D'Elia A.V., Driul L., Giacomello R., et al. Frequency of factor V, prothrombin and methylenetetrahydropholate reductase gene variants in pre-eclampsia. Gynecol Obstet Invest 2002; 53: 84-87.
24. Benedetto C., Marozio L., Salton L., et al. Factor V Leiden and factor II G20210A in pre-eclampsia and HELLP syndrome. Acta Obstet Gynecol Scand 2001; 81: 1095- 1100.
25. Pavlova E., Chemev T., Chemev A., et al. Pregnancy and issues with inherited and aquired thrombophilia. Journal of IMAB- Annula Proceeding 2008; 21-22.
26. Omar S.Z., Qvist R., Khaing Si L., et al. Thrombophilic mutations in pre-eclampsia and pregnancy- induced hypertension. Journal of Obstetrics and Gynaecology Research 2008; 34 (82): 174- 178.
27. Makatsariya A.D., Bitsadze V.O., Baimuradova S.M., et al. Prevention of recurrent preeclampsia in women with thrombophilia. Journal of Thrombosis and Haemostasis, vol 7 Suppl 2, Abstracts from XXII ISTH Congress, 2009.
28. Mello G., Parretti E., Marozio L., et al. Thrombophilia is significantly associated with severe preeclampsia. Results of large scale, case- controlled study. Hypertension 2005; 46: 1270- 1274.
29. Driul L., Damante G., D'Elia A., et al. Genetic thrombophilias and uterine artery Doppler velocimetry and preeclampsia. International Journal of Gynecology Obstetrics 2005; 88 (3): 265- 270.
30. Best L., Dorsam S., Nadeau M., et al. Genetic thrombophilia variants and the risk for preeclampsia among American Indians. Hypertension and pregnancy 2009; 28 (1): 85-94.
31. Livingston J.C., Barton J.R., Park V., et al. Maternal and fetal inherited thrombophilias are not related to the development of severe preeclampsia. Am J Obstet Gynecol 2001; 185: 153-157.
32. Hauge B., Wiltshire E., Nelson P., et al. Polymorphisms of methylenetetrahydropholate reductase in women with pre-eclampsia 12th international Society for the study of hypertension in pregnancy. Hypertens Pregnancy 2000; 19: 23.
33. Kupferminc M.J., Peri H., Zwang E., et al. High prevalence of the prothrombin gene mutation in women with intrauterine growth retardation, abruptio placentae and second trimester loss. Acta Obstet Gynecol Scand 2000; 79: 963-967.
34. von Tempelhoff G.F., Heilmann L., Spanuth E., et al. Incidence of factor V Leiden mutation, coagulation inhibitors deficiency, and elevated antiphospholipid antibodies in patients with preeclampsia or HELLP syndrome. Thromb Res 2000; 100: 363-365.
35. Preston F.E., Rosendaal F.R., Walker I.D., et al. Increased fetal loss in women with heritable thrombophilia. Lancet 1996; 348: 913-6.
36. Lin J., August P. Genetic thrombophilias and preeclampsia. A meta-analysis. Obstet Gynecol. 2005; 105: 182-192.
37. Schlembach D., Beinder E., Zingsem J., et al. Association of maternal and/or fetal factor V Leiden and G20210A prothrombin mutation with HELLP syndrome and intrauterine growth restriction. Clin.Sci 2003; 105: 279- 285.
38. Hayashi M. Aetiology of pre-eclampsia and thrombophilic genetic mutations. Clinical Science 2003; 105: 269- 271.
39. Vefring H., Lie R.T., Degard R., et al. Maternal and fetal variants of genetic trombophilia and the risk of preeclampsia. Epidemiology 2004; 15 (83): 317-22.
40. Ariel I. Placental pathology in fetal thrombophilia. Hum Pathol 2004; 35 (5): 729-33.