Association Between Thrombophilia Gene Polymorphisms and Recurrent Pregnancy Loss

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Spontaneous miscarriage is reported in approximately 15% of the clinically recognized pregnancies. Several reports have showed an increased risk of miscarriage in patients with thrombophilia, but due to the heterogeneity of study design the role thrombophilic factors and the use of anticoagulant therapy in prevention of pregnancy loss is still unclear. The current study includes 55 patients for which we ran a screening of the most commonly inherited thrombophilia mutations (FVL, FII, MTHFR C677T/A1298, PAI 4G/5G mutations). We found that most of the patients (92.72%) associated mutation in at least 2 of the genes evaluated. Only a small number of patients (7.27%) had a single variant identified. We have found high prevalence of the studied variants in the pregnant patients that experience pregnancy loss, with risk allele frequencies increased from 2 to 11 times as compared to the general population. We consider that evaluation of the thrombophilic variants should be indicated for patients with pregnancy loss in order to establish a possible cause for the miscarriage.

Keywords: pregnancy, thrombophilia, chromosomal abnormalities, miscarriage, karyotype

Spontaneous miscarriage is reported in approximately 15% of the clinically recognized pregnancies [1]. Among the causes of pregnancy loss, there are three in particular considered as major factors, including: chromosomal abnormalities, inflammatory and autoimmune disorders [2-4], and polymorphisms of pro-thrombophilic genes [5, 6]. The carrier of chromosomal abnormalities are at risk of producing defective gametes and were found to have a higher rate of repeated miscarriages as compared with patients with normal karyotype [7, 8].

Several reports have shown increased risk of miscarriage in patients with thrombophilia, but due to the heterogeneity of study design the role thrombophilic factors and the use of anticoagulant therapy in prevention of pregnancy loss there is still controversial [9, 10].

It is known that the pregnancy outcomes influenced by the utero-placental circulation because the obstruction in the placental vessels will lead to pregnancy complications, in particular miscarriage.

In addition to the hypercoagulable state of pregnancy, in cases of thrombophilia there is an increased risk to induce placental thrombosis and placental insufficiency which will end up in obstetrical complications [11-14].

Moreover, in recurrent miscarriage identifying the causes is very important for a better couple management and preventing depression [15].

Experimental part

We have done a prospective study of the medical records of preg-nant patients, who were admitted to the Obstetrics and Gynecology Clinic Dumitru Popescu Timisoara between 2014 and 2017. Pregnant patients with history of early miscarriage were included in the study. We excluded the cases with known cause of pregnancy loss, cases with abnormal fetal karyotype, and carriers of chromosomal aberrations with chronic or acute immune/infectious diseases and uterine anomalies. A total of 55 patients met the inclusion criteria and we ran a screening of the most commonly inherited thrombophilia mutations (FVL, FII, MTHFR C677T/A1298, PAI 4G/5G mutations). We noted the age of each patient, gravidity, parity, previous abortions, and live births, as well as the gestational age of the last miscarriage.

The study group included patients with one or more miscarriages, at least one type of mutation of the screened mutations, and no anticoagulant therapy during the previous pregnancies.

The study was approved by the Ethics Committee of the Victor Babes University of Medicine and Pharmacy Timisoara, in accordance to the Helsinki Declaration and to some published models [16–18].

The screening of Factor V Leiden, FII, MTHFR C677T/A1298C and PAI 4G/5G gene poly-morphisms were analyzed by polymerase chain reaction. Molecular analysis was conducted in the Center for Genomic Medicine from the University of Medicine and Pharmacy Timisoara. From each patient that met the inclusion criteria were collected 2 mL venous blood in EDTA tubes. We used the Gene Proof Genotyping PCR kits for detection of the above mentioned polymorphism and the experiments were done on Real Time PCR Platform Light Cycler 480 according to the kits manufacturer indications.

We performed statistical analysis using IBM SPSS Statistics 23 program and a two-tailed p value < 0.05 was considered significant. To describe the cohort, we tested the data normality distribution. We calculated expected value and we considered a cut-off point of 5. Chi2 test or Fisher's Exact test were used to compare proportions.

Results and discussions

The maternal age varied between 18 and 41 years, with a mean age of 32.02 ± 5.201 years. The mean gestational age at the time of abortion varied between 6 and 14 weeks, with a mean of 8.15 ± 2.27 weeks.

Among the cases, 54 (98.18%) had zero parity and only one case (1.81%) had parity 2. From the 55 cases included

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Table 1
THE PREVALENCE OF GENOTYPES AND ALLELES FREQUENCIES IN THE STUDY LOT

Gene	dbSNP ID	Genotype (%)			Risk allele	MAF Risk allele	MAF in study
MTHFR C677T	rs1801133	CC 27.3	CT 56.4	TT 16.4	T	36.4%	89.2%
MTHFR A1298C	rs1801131	AA 41.8	AC 54.5	CC 3.6	C	31.3%	61.7%
FVL	rs6025	GG 85.5	GA 14.5	AA 0	A	1.1%	14.5%
FII (G 20210A)	rs1799963	GG 90.9	GA 9.1	AA 0	A	0.8%	9.1%
PAI-1 4G	rs1799889	5G5G 1.8	5G4G 76.4	4G4G 21.8	4G	There is no frequency data	

in the study lot, 42 (76.36%) had positive history of previous miscarriages, 25 patients (45.45%) had 1 abortion, 12 patients (21.81%) had 2 abortions, 2 patients (3.63) had 3 miscarriages and 3 patients (5.45%) had 4 previous abortions.

The prevalence of the genotypes and alleles frequencies is presented in table 1. MTHFR C677T variant was found in heterozygosity in 56.4% of the patients, while 16.4% of the cases had the homozygote TT genotype. From the total of the patients, 54.5% presented the AC genotype for MTHFR A1298C and 3.6% had the CC genotype. FVL mutation was present only in heterozygote state, 14.5% of the patients exhibit the GA genotype. For the FII gene, was recorded a prevalence of 9.1% for heterozygote state, and no case of homozygosity FII. The most prevalent genotype for PAI-1 gene was 5G4G - 76.4%, the prevalence of homozygotes was 21.8%.

Considering the importance of establishing the cause of miscarriage, we investigated the prevalence of the polymorphisms in the genes related to thrombophilia for the patients that had no chromosomal aberrations detected in the aborted fetus. In the study of Paidas et al. showed that the recurrence rate of obstetric complications for the patients without thrombophilia is about 23%, but for cases presenting polymorphisms in the genes associated with thrombophilia there is an increased risk of adverse pregnancy outcome. There are many studies conducted in patients with thrombophilia and recurrent abortions that had the aim to improve maternal and fetal perinatal outcomes [10, 19, 20].

During fetal development, folate is essential for the proper development of fetus in the first trimester of the pregnancy. Before and shortly after conception is recommended an increased folate intake for rapid growth of the uterus and the placenta, as well as for the development of the fetus [21-24].

Aberrations in folate pathway were found to contribute to the etiology of recurrent abortions in different populations. MTHFR plays an essential role in the folate metabolism, catalyzing the conversion of 5, 10-methylenetrahydrofolate to 5-methylenetrahydrofolate that is needed for methionine formation. There are two functional polymorphisms in the MTHFR gene-C677T and A1298C that have been associated with reduced enzyme activity of MTHFR [25, 26].

MTHFR A1298C polymorphism is not responsible for an increase of total homocysteine, but it contributes to the increase of homocysteine levels [27]. In the present study, the AC and CC genotypes were found in 58.1% of the patients. We have found a significant correlation between the presence of *MTHFR A1298C* and the number of abortions ($p = 0.004$). The risk allele frequency was almost double when compared with frequency in the general population, (61.7% vs 31.3%).

There are several studies regarding the association of *MTHFR C677T* variant with an increased risk of miscarriage. The prevalence of MTHFR C677T variants varies among the populations, in Europe, the highest T allele frequency is found in Mediterranean region, while the northern countries have the lowest T allele frequency [28]. In the present study, 72.8% of the patients exhibit the risk allele T, in homozygous or homozygous state. We could not find a significant statistical association between the *MTHFR C677T* polymorphism and the number of abortions ($p > 0.05$). These is in line with other studies that showed that *MTHFR C677T* polymorphism was associated with the increased risk of miscarriage in Asians, but not in Caucasians [29, 30]. The risk allele frequency in the present study was more than double than the risk allele frequency in the general population (89.2% vs 36.4%).

In two cases, a very rare combination of genotypes was found, one case exhibited the 677CT/1298CC and the second case presented the 677TT/1298AC genotype. Two studies evaluating the association of *C677T* and *A1298C* MTHFR polymorphisms suggested a decreased viability among subjects carrying these mutations. They indicated a possible disadvantageous selection among fetuses with increased numbers of MTHFR polymorphisms, rising the hypothesis that the effect of MTHFR mutated alleles would be detrimental during embryogenesis when high levels of folate are needed [31, 32]. Unfortunately, DNA samples from the aborted fetuses were not available in order to evaluate de fetuses' genotypes.

Factor V Leiden (FVL) was hypothesized to be one of the leading factors that indicates a poor pregnancy outcome [33-35]. There are several studies showing a positive correlation between FVL and an increased risk of miscarriage, while other reports could not establish any significant association between FVL mutation and recurrent fetal loss [36].

FVL results from a guanine to adenine substitution at nucleotide 1691 in the FV gene which lead to a substitution of arginine by glutamine at position 506. FVL mutations occur in 3 to 5% of the general population and encodes for a protein resistant to degradation by the protein C, impairing the signaling for anticoagulation and fibrinolysis [37, 38]. In the current study, 14.5% of the patients presented the FVL in heterozygote state, no case with homozygous mutation was found. The prevalence of the mutation in the study lot is above the values found in the general population, and similar with the results from Fokka's report following the FVL in relation with the risk of pregnancy loss in Greek population. The risk allele frequency in the study lot is 13 fold increased as compared to the general population (14.5% vs 1.1%).

Prothrombin (FII) G20210A mutation results from a substitution of guanine to adenine at position 20210 in the untranslated region of prothrombin gene. FII effect is an

increase of mRNA and of prothrombin levels by 133% contributing to the hypercoagulability state [39, 40]. FII G20210A is considered the second most frequent cause of thrombophilia and has a prevalence of 1-4% in the general population, but there are geographical variations: in Southern Europe the prevalence is 3 %, in Northern Europe - 1.7 %, in Asian and African descent, innative Americans and in Australians is very rare [41].

There are several reports showing a link between prothrombin mutation and adverse obstetric events, but Silver's study done on a large cohort failed to prove a correlation between prothrombin mutation and an increased risk of miscarriage, preeclampsia, intrauterine growth restriction, placental abruption [42-44]. In the current study, the prothrombin mutation was found in heterozygote state in 9.1% of the patients, risk allele frequency being more than 11 fold increased as the allele frequency reported in the general population (9.1% vs 0.8%).

PAI-1(or serpin E1) is a member of the superfamily of serine-protease inhibitors and is the principal inhibitor of both the tissue-type plasminogen activator (t-PA) and the urinary-type plasminogen activator (u-PA). During pregnancy PAI-1 is the primary plasmatic inhibitor of tPA [45]. 4G/5G polymorphism in the PAI-1 gene has been correlated with levels of plasma PAI-1 [46]. It has been suggested that the 4G allele results in higher activity than the 5G allele, and has been linked to an increased risk for venous thromboembolism, coronary disease, severe preeclampsia, abruptic placentae, and some other obstetric complications [47-49]. Even though some studies found no association between these variants and pregnancy outcome recent reports showed o positive correlation between 4G allele frequency and miscarriage [50, 51]. The prevalence of this variant in specific populations as well as the risk allele frequency is not yet enough studied and large cohort studies are still needed. In the present study we have found a high prevalence of the 4G variant, 76.4% of the patients presented the heterozygote genotype 4G5G, while 21.8% of the patients were homozygotes and exhibit only the 4G variant (4G4G).

When analyzing the number of variants found in the study lot, we found that most of the patients (92.72%) associated mutation in at least 2 of the genes evaluated, as showed in Figure 1. Only a small number of patients (7.27%) had a single variant identified. Our results show a high prevalence of trombophilic defects in the study lot as compared with the results from the other studies where combined thrombophilic defects were documented in 21% of women with pregnancy loss compared with 5.5% of control patients [52-55].

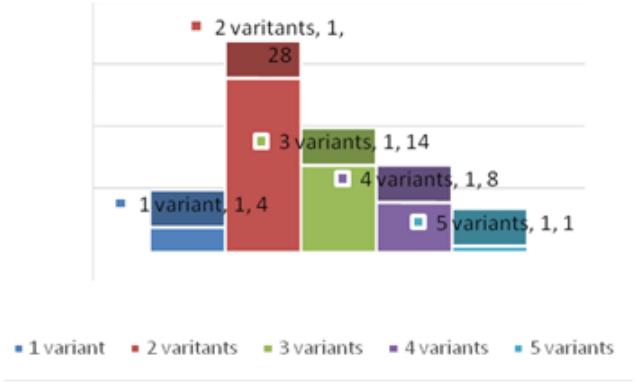

Fig. 1. Number of variants found for each patient from the study lot

Conclusions

As of our knowledge this is the first study evaluating the MTHFR C677T, MTHFR A1298C, FVL, FII (G 20210A), PAI-1 4G polymorphism associated with thrombophilia in a lot of pregnant women from the Western part of Romania. We have found high prevalence of the studied variants in the pregnant patients that experience pregnancy loss, with risk allele frequencies increased from 2 to 11 times as compared to the general population. We consider that evaluation of the trombophilic variants should be indicated for patients with pregnancy loss in order to establish a possible cause of miscarriage.

Miscarriages represent a significant emotional burden not only for women, but also for their partners. Psychological counselling, in addition to medical investigations and consultation, is beneficial in reducing women's distress after miscarriage. The emotional distress of the patients can be better managed if the cause of miscarriage is identified. By evaluating the possible causes of a miscarriage, a better management of the couple is possible, but it can also contribute to the prevention of depression for the patients that had one or more pregnancies loss.

Acknowledgments: This research was done in the Center of Genomic Medicine from the Victor Babes University of Medicine and Pharmacy, Timi°oara, POSCCE Project ID: 1854, cod SMIS: 48749, "Center of Genomic Medicine v2", contract 677/09.04.2015.The research was done thorough the collaboration established under the PNCDI III project, BM 29/2016.

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Manuscript received: 23.03.2018