Ethnic Differences in the Association of Thrombophilic Polymorphisms with Obstetric Complications in Slovak and Roma (Gypsy) Populations

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Aims: Hereditary as well as acquired thrombophilia is associated with a higher incidence of severe obstetric complications such as preeclampsia, spontaneous pregnancy loss, placental abruption, and fetal growth retardation. The aim of our study was to examine the association of selected thrombophilic polymorphisms (factor V Leiden, *MTHFR* C677T, and *MTHFR* A1298C) with pregnancy complications in the Slovak majority population and the Roma (Gypsy) ethnic population. The study included 354 women; 120 patients and 105 controls from the Slovak majority population, 50 patients and 79 controls from the Slovak Roma population. Genotyping was performed by the real-time polymerase chain reaction method using TaqMan[®] MGB probes. **Results:** A statistically significant higher frequency of factor V Leiden ($p=0.001$, odds ratio [OR]=5.9) and *MTHFR* C677T polymorphism ($p=0.011$, OR=1.7) was observed in the Slovak majority patient group compared to the control group. The incidence of *MTHFR* A1298C polymorphism between patients and controls did not differ significantly. None of the three polymorphisms studied was in association with pregnancy complications in the group of Roma women. **Conclusions:** Our study has confirmed the variable distribution of selected thrombophilic polymorphisms in different ethnic groups as well as their various effects on the clinical phenotype.

Introduction

INHERITED THROMBOPHILIAS ARE a heterogeneous group of coagulation disorders that predispose individuals to thrombosis; the most common causes include factor V Leiden, prothrombin G20210A gene mutations, methylenetetrahydrofolate reductase (*MTHFR*) gene mutations, antithrombin III deficiency, and protein C and S deficiencies (Oqueh *et al.*, 2001).

Hereditary thrombophilia has gained a lot of attention as a risk factor for pregnancy complications in recent years. Inherited thrombophilia along with the physiological hemostatic changes during pregnancy may shift the fine balance toward thrombotic changes in the placenta, which leads to inadequate fetomaternal circulation and consequently to a decrease in placental perfusion (Hoffmann *et al.*, 2012). These disturbances in coagulation may contribute to the etiology of serious pregnancy complications such as preeclampsia, fetal growth restriction, stillbirth, and placental abruption that represent important contributors to both perinatal and maternal morbidity and mortality (Kupferminc *et al.*, 2000).

One of the forms of hereditary thrombophilia is factor V Leiden, a common mutation G → A in the nucleotide position 1691 in the *factor V* gene, which is associated with a significantly increased risk for systemic venous thrombosis (Dahlbäck, 1995). Epidemiological studies suggest that inherited and acquired thrombophilia of the mother, such as that caused by the Leiden polymorphism in blood coagulation factor V, contributes to the pathogenesis of fetal loss as well as other adverse pregnancy outcomes (Kovalevsky *et al.*, 2004; Facco *et al.*, 2009). The *MTHFR* gene C677T and A1298C polymorphisms influence the metabolism of homocysteine and folic acid. The exact relationship between *MTHFR* polymorphisms and hyperhomocysteinemia is still unclear. Several studies have confirmed the influence of *MTHFR* polymorphisms on increased levels of plasma homocysteine (Chambers *et al.*, 2000; Yakub *et al.*, 2012), and hyperhomocysteinemia has been associated with pregnancy loss and other obstetric complications (Facco *et al.*, 2009).

A large number of case-control studies have reported associations between adverse pregnancy outcomes and inherited thrombophilias, but the results have been heterogeneous. Some studies were also unable to answer questions regarding the

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association between inherited thrombophilias and pregnancy complications, because they did not account for the potential interaction of other coinherited thrombophilias, as well as for specific prevalence of inherited thrombophilias in studied ethnic groups (Said, 2011).

The aim of our study was to examine the association of three thrombophilic polymorphisms (factor V Leiden, *MTHFR* C677T, and *MTHFR* A1298C) with selected pregnancy complications in a group of women of the Slovak majority population as well as in the group of Roma (Gypsy) women. This research was motivated by the lack of information on this topic in Slovakia and the complete absence of any data for the Roma population.

Materials and Methods

The study included 354 women (16–46 years, average age 27.96 ± 6.48 years) hospitalized at the Department of Gynecology and Obstetrics, J. A. Reiman Faculty Hospital in Prešov in the years 2007–2011. All probands had signed an informed consent and all institutional ethics requirements were met.

The group of patients consisted of 170 women (120 of Slovak majority population and 50 of Slovak Roma population) with selected obstetric complications. From the total number of patients, 91 women (53.53%) experienced spontaneous pregnancy loss in the second or third trimester; of that, 66 women (38.82%) had a history of one pregnancy loss and 25 women (14.71%) had two or more pregnancy losses. Seventy-nine women (46.47%) were hospitalized with other pregnancy complications—intrauterine growth retardation, preeclampsia, placental abruption, or deep vein thrombosis. Patients with known causes of pregnancy loss, for example, chromosomal abnormalities, were excluded from the study.

The control group consisted of 184 age-matched women with no previous history of pregnancy complications (105 of Slovak majority population and 79 of Roma population).

Genomic DNA was extracted from buccal swabs with the DNA extraction kit JetQuick (Genomed GmbH, Löhne, Germany) according to the standard procedure. Factor V Leiden, *MTHFR* C677T, and *MTHFR* A1298C polymorphisms were detected by real-time polymerase chain reaction (PCR) using fluorescent labeling of the amplified segment with TaqMan[®] MGB probes. Genotyping was performed on 7500 Fast Real-time PCR System (Applied Biosystems, Foster City, CA).

Allele frequencies were calculated by the gene-counting method from the observed genotypes. Comparisons of allele and

genotype differences were tested by Pearson's goodness-of-fit chi-square ($df = 1$), and the significance level was set at $p < 0.05$.

Results and Discussion

Genotype and allele frequencies for factor V Leiden are shown in Table 1. The frequency of factor V Leiden was significantly higher in patients (7.9%) than in controls (1.4%) in the group of Slovak majority women ($p = 0.001$), and according to the odds ratio (OR), the presence of factor V Leiden allele is associated with a nearly sixfold higher risk of pregnancy complications in patients of the Slovak majority population. Similarly designed studies from Slovakia are not available, so we compared our results with the ones from the neighboring Czech Republic. The importance of screening for thrombophilic polymorphisms in the group of women after the first early pregnancy loss in the Czech population was evaluated by Kasparova *et al.* (2012). The heterozygous form of factor V Leiden occurred 1.8 times more frequently in the group of patients compared to the general Czech women population. However, Subrt *et al.* (2008) did not confirm the association of factor V Leiden with recurrent pregnancy loss in Czech patients. An extensive association study (Lykke *et al.*, 2012) found out that factor V Leiden increased the risk of severe preeclampsia (OR = 1.6), fetal growth restriction (OR = 1.4), and placental abruption (OR = 1.7). Several studies dealing with pregnancy loss and thrombophilia were summarized in meta-analyses (Rey *et al.*, 2003; Dudding and Attia, 2004), which describe the increased level of risk with $OR \geq 2$ for carriers of factor V Leiden. On the other hand, factor V Leiden association with pregnancy complications was not confirmed by two prospective studies (Lindqvist *et al.*, 1999; Murphy *et al.*, 2000).

Our analysis indicates a high frequency of factor V Leiden in the control group of Roma women with an allele frequency of 7%. This result is congruent with other studies showing that healthy asymptomatic Hungarian (Balogh *et al.*, 1999) and Slovak (Bozikova *et al.*, 2012) Roma ethnic populations have a high-factor V Leiden frequency. The studies on the occurrence of pregnancy complications in the Roma population reported a higher incidence of intrauterine growth retardation and pregnancy loss compared to the majority populations of the given countries (Rimarova *et al.*, 2004; Bobak *et al.*, 2005). Therefore, we expected the frequency of thrombophilic factors in a group of Roma patients to be higher compared to the control group. On the contrary, the frequency of allele A was even slightly higher in the control

TABLE 1. FREQUENCY OF FACTOR V LEIDEN IN PATIENT AND CONTROL GROUPS

Factor V Leiden	Patients	Controls	Patients	Controls
	Slovak population (n = 120)	Slovak population (n = 105)	Roma population (n = 50)	Roma population (n = 79)
1691 GG	102 (85.00%)	102 (97.14%)	44 (88.00%)	69 (87.34%)
1691 GA	17 (14.17%)	3 (2.86%)	6 (12.00%)	9 (11.39%)
1691 AA	1 (0.83%)	0	0	1 (1.27%)
f (A)	7.9%	1.4%	6.0%	7.0%
OR (95% CI)	5.932 (1.730–20.342)		0.853 (0.305–2.384)	
p	0.001		0.762	

CI, confidence interval; OR, odds ratio.

TABLE 2. FREQUENCY OF *MTHFR* C677T IN PATIENT AND CONTROL GROUPS

<i>MTHFR</i> C677T	<i>Patients</i>	<i>Controls</i>	<i>Patients</i>	<i>Controls</i>
	<i>Slovak population</i> (n = 120)	<i>Slovak population</i> (n = 105)	<i>Roma population</i> (n = 50)	<i>Roma population</i> (n = 79)
677 CC	47 (39.17%)	56 (53.33%)	31 (62.00%)	45 (56.96%)
677 CT	50 (41.67%)	38 (36.19%)	12 (24.00%)	27 (34.18%)
677 TT	23 (19.16%)	11 (10.48%)	7 (14.00%)	7 (8.86%)
f (T)	40.0%	28.6%	26.0%	25.9%
OR (95% CI)	1.667 (1.122–2.475)		1.003 (0.566–1.775)	
<i>p</i>	0.011		0.993	

group (7%) than in the group of patients (6%), although the difference was not statistically significant ($p=0.762$). It would be useful to confirm these results by functional studies and explore the possibility that the Roma population has some kind of protective factor that mitigates the procoagulant effect of factor V Leiden. Our results are in agreement with a study from northern India (country of origin of the Roma population) that reported no significant difference in factor V Leiden frequency in the group of women diagnosed with recurrent pregnancy loss and healthy controls (Mukhopadhyay *et al.*, 2009).

The *MTHFR* 677T allele had a significantly higher frequency in patients (40%) than in controls (28.57%) of Slovak majority population, $p=0.011$ (Table 2). According to the OR, women with 677T allele have a 1.7 times higher risk of occurrence of pregnancy complications. *MTHFR* 677T was associated with placental infarcts and subsequent fetal growth restriction (Hefler *et al.*, 2004; Lin and August, 2005). In the study from Lykke *et al.* (2012), *MTHFR* C677T was significantly associated only with severe preeclampsia (OR = 1.3). Nair *et al.* (2012) reported strong association of this polymorphism with recurrent pregnancy loss in the population from northern India. The association of *MTHFR* 677TT genotype with recurrent pregnancy loss was not confirmed by studies from Makino *et al.* (2004) and Biswas *et al.* (2008).

There was no difference in *MTHFR* C677T allele frequencies in the groups of patients and controls of Roma ethnicity. This polymorphism probably does not affect the risk of occurrence of pregnancy complications in the Roma population. Interesting results were reported by Gasparovic *et al.* (2004) on the levels of plasma homocysteine and *MTHFR* C677T polymorphism in the Slovak and Roma population groups. Distribution of *MTHFR* C677T genotypes in both populations was not significantly different, but the homocysteine levels were significantly lower in the group of

Roma; TT homozygosity significantly increased homocysteine levels only in the Slovak majority population. This polymorphism seems to have no effect on the level of plasma homocysteine in the Roma ethnic group; thus, it is probably not a relevant marker for estimating the risk of pregnancy complications associated with thrombophilia in this population. A higher level of plasma homocysteine is also affected by the lack of vitamins B6, B12, and folic acid. The average intake of vitamins in the diet of Roma, among them vitamin B6 and folic acid, is below the recommended daily dosage (Sivakova *et al.*, 2007). Krajcovicova-Kudlackova *et al.* (2004) observed significantly lower plasma levels of those vitamins in the Roma population in comparison with the Slovak population, but the homocysteine levels were similar in both populations. Normal homocysteine levels in the Roma population in concurrence with a higher incidence of deficiency of vitamin B6 and folic acid, smoking, and high alcohol consumption suggest more efficient metabolism of homocysteine in relation to different ethnic origins.

The frequency of *MTHFR* A1298C did not significantly differ in our studied groups (Table 3), this polymorphism seems to have no effect on the incidence of examined pregnancy complications, and its occurrence was not affected by ethnicity either. Subrt *et al.* (2008) indicated that the frequency of *MTHFR* C677T and A1298C in the group of 206 patients with recurrent pregnancy loss did not differ from the asymptomatic Czech population. Infante-Rivard *et al.* (2002) observed a reduced risk of intrauterine growth restriction in mothers who were homozygous carriers of the *MTHFR* A1298C polymorphism. Bogdanova and Markoff (2010) suggest optional testing of *MTHFR* A1298C polymorphism only in conjunction with C677T, and its relevance should be judged with caution.

Given the smaller group of tested patients of Roma ethnicity compared to the group of patients from the Slovak majority

TABLE 3. FREQUENCY OF *MTHFR* A1298C IN PATIENT AND CONTROL GROUPS

<i>MTHFR</i> A1298C	<i>Patients</i>	<i>Controls</i>	<i>Patients</i>	<i>Controls</i>
	<i>Slovak population</i> (n = 120)	<i>Slovak population</i> (n = 105)	<i>Roma population</i> (n = 50)	<i>Roma population</i> (n = 79)
1298 AA	54 (45.00%)	42 (40.00%)	18 (36.00%)	28 (35.44%)
1298 AC	54 (45.00%)	51 (48.57%)	26 (52.00%)	40 (50.63%)
1298 CC	12 (10.00%)	12 (11.43%)	6 (12.00%)	11 (13.93%)
f (C)	32.5%	35.7%	38.0%	39.2%
OR (95% CI)	0.867 (0.586–1.281)		0.949 (0.567–1.588)	
<i>p</i>	0.473		0.842	

population, we tried to exclude the possibility of familial kinship between probands through evaluating their personal data and removing potential relatives from the studied group. Taking into account that the Roma population in Slovakia represents a genetically isolated population, characterized with a high frequency of consanguinity and inbreeding (Ferak *et al.*, 1987), this aspect could affect the outcome of our study.

As we already mentioned, the incidence of pregnancy complications is higher in the Roma population compared to the majority populations of given countries (Rimarova *et al.*, 2004; Bobak *et al.*, 2005). The increased risk of spontaneous abortion as well as stillbirth is generally associated with low socioeconomic status (Stephansson *et al.*, 2001; Norsker *et al.*, 2012). The Roma minority has, with very few exceptions, a low socioeconomic status and educational background, and together with unhealthy dietary habits, poor hygiene, and low maternal age, this could be the possible cause of negative obstetric outcomes. This is supported by the study of Bobak *et al.* (2005) claiming that differences in birth outcomes between Roma and non-Roma mothers in the Czech Republic are to a considerable extent determined by socioeconomic factors.

In conclusion, our study has shown the association of factor V Leiden and *MTHFR* C677T, but not *MTHFR* A1298C polymorphism, with pregnancy complications in women of the Slovak population. None of the three polymorphisms studied was in association with pregnancy complications in a group of Roma women. Ethnicity appears to be a confounding factor when examining the association between thrombophilia and obstetric complications. Therefore, when testing for inherited thrombophilias, the ethnic origin of the patient needs to be considered, as suggested also by Bennett *et al.* (2012).

Acknowledgments

This work was supported by the grants APVV-0716-10 funded by the Slovak Research and Development Agency and ITMS 26220120023 funded by the European Regional Development Fund.

Author Disclosure Statement

No competing financial interests exist.

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