The C677T Variant in the Methylentetrahydrofolate Reductase Gene and Idiopathic Spontaneous Abortion in a Romanian Population Group

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Abstract

Spontaneous abortions (SA) are a major public health problem and a frequent pregnancy associated disorder. Hereditary thrombophilia and hyperhomocysteinemia are considered to be important factors altering the placental circulation, the in utero development and the evolution of pregnancy. The MTHFR gene (methylentetrahydrofolate reductase) exhibits an intensely studied polymorphism, C677T, that was repeatedly associated with hyperhomocysteinemia, increased thrombotic risk and was studied in relation with SA susceptibility. This study was aimed to assessing the correlation of this polymorphism with idiopathic sporadic or recurrent SA in a Romanian population. In the case-control study, 131 patients with a history of SA and 135 women with no SA and at least one uneventful term delivery were included. The PCR-RFLP technique (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) was used to genotype the cases and controls and the results were analysed using the χ2 test. The present analysis indicates that the MTHFR 677TT homozygous genotype is positively associated with recurrent idiopathic SA (OR 2.493, 95%CI 0.974, 6.379, p-value 0.06). This association was no longer observed in sporadic SA patients (OR 1.214, 95%CI 0.488, 3.017, p-value 0.814). In conclusion, the present study is consistent with previous reports which state that the presence of MTHFR 677T variant in homozygous status could represent a genetic susceptibility factor for recurrent idiopathic SA. Moreover, this is the first attempt to investigate this correlation in a Romanian group and to offer epidemiological support in estimating the frequencies of some common genetic variants in the Romanian population.

Keywords: association, folates, homocysteine, polymorphism, pregnancy

Introduction

At present, reproductive failure is one of the major public health problems. In humans, the probability of conception during one menstrual cycle is approximated at a maximum of 40% (Kavalier et al., 2005). Moreover, 10-15% of clinically recognized pregnancies end in spontaneous abortion (SA). The causes of this can be fetal or maternal, anatomical, infectious, immunological or genetic, and in approximately 50% of cases, despite all investigations, the causes cannot be determined. In this latter situation, the SA is declared idiopathic (Carp et al., 2004; Kavalier et al., 2005 Royal College of Obstetricians and Gynecologists, 2003).

Over the past years, emerging information has described that pregnancy-associated thrombophilia could be a factor altering the normal evolution of pregnancy. Numerous studies focus on the mechanism through which hyperhomocysteinemia, hereditary thrombophilia (such as protein C or protein S deficiency, the factor V Leiden mutation, the prothrombin G20210A mutation) or combinations of these factors, could influence the normal in utero development and even play a role in spontaneous abortion etiology. Some authors suggest that as many as 60% of pregnancy losses could have been provoked by coagulation disorders (Bick and Hoppensteadt, 2005; Mitic et al., 2010). However, these conclusions are often inconsistent and results are contradictory (Altomare et al., 2007; Robertson et al., 2005; Rodger et al., 2010). Considering the subject of deep venous thrombosis, the most studied mutations in hereditary thrombophilia are the Leiden mutation, the G20210A mutation of prothrombin (factor II) and the variants of the MTHFR (methylentetrahydrofolate reductase) gene. Also in relation with pregnancy and SA, the aforementioned variants are most extensively investigated in association studies (Coulam et al., 2008; Kovalesky et al., 2004). MTHFR is an extremely important enzyme in the homocysteine and folate metabolism which catalyses the production of 5-methyltetrahydrofolate. This compound is the methyl donor for the metabolism of homocysteine to methionine and, thus, the reduction of plasma homocysteine levels (Reyes-Engel et al., 2002). Several variants of this gene can alter the activity of the MTHFR enzyme, the two best known of these being

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the MTHFR C677T and A1298C single nucleotide polymorphisms (SNP) (Frosst et al., 1995; Van der Put et al., 1998). Hyperhomocysteinemia is recognised as a thrombosis risk factor exerting its detrimental effects through diverse mechanisms (Brattstrom et al., 1998; Cetin et al., 2010; Nadir et al., 2007). The MTHFR C677T variant has already been correlated with high homocysteine and low folic acid levels in various populations and pathologies (Altomare et al., 2007; Bagheri et al., 2010; Gudnason et al., 1998; Gueant-Rodriguez et al., 2006).

The aim of the present study is to investigate the distribution of the MTHFR C677T polymorphism as a possible genetic risk factor for idiopathic SA in a Romanian population group.

Materials and methods

The present research was designed as an observational case-control study. Between March 2007 and December 2009 for examinations, genetic counseling or other investigations, 266 Caucasian women from the Romanian population were recruited from the patients visiting the office of the IIth Genetic Investigations Laboratory, Cluj-Napoca, Romania. The case group consisted of 131 fertile age women with a history of first trimester SA (first 14 weeks of gestation), declared idiopathic after clinical and paraclinical investigations: ultrasonography, hormonal dosage, antiphospholipid antibodies dosage, TORCH serology, karyotype analysis. For the analysis of risk, the case group was further divided in two subgroups depending on the number of SA events: 46 cases with recurrent SA (at least 3 SA) and 85 cases with history of less than 3 SA. The control group was made of 135 women with at least one uncomplicated pregnancy and the birth of a healthy child, with no history of spontaneous abortion. The participation in this study was voluntary and written informed consent was obtained from all participants upon inclusion. A set of general parameters of the study groups is presented in Tab. 1.

For the genotype analysis, 5 ml samples of peripheral blood were harvested on EDTA and stored at 4°C until DNA isolation. DNA was extracted out of white blood cells using Wizard Genomic DNA Purification Kit (Promega, MA, USA). The quantity and purity of the DNA samples were analysed using an Eppendorf photometer (Eppendorf, Germany). A DNA concentration of 50-100 ng/μl was considered satisfactory for the analysis. The detection of the MTHFR C677T SNP was performed through the PCR-RFLP technique (polymerase chain reaction-restriction fragment length polymorphism) after the protocol described by Zhou-Cun et al. (2006), with some modifications. The following primers were used to amplify a 265 bp fragment containing the polymorphism: Fw 5’-CATCCTATTGGCAG GTT AC-3’ and Rev 5’-GACGGTGGCGGTAG AGTG-3’. The amplification took place in 25 μl of reaction mix containing: 12.5 μl PCR Mix (recombinant Taq polymerase 0.05 IU/μl; MgCl₂ 4 mM; dATP, dGTP, dCTP, dTTP at a concentration of 0.4 mM each), 1 μl BSA solution (Bovine Serum Albumine) 2 mg/ml, 1 μl of each primer solution (1.5 pmoles/μl), 2 μl of DNA sample and nuclease-free water until the final volume of 25 μl. The PCR consisted of an initial denaturation of 5 minutes at 94°C followed by 35 amplification cycles (30 seconds-94°C, 30 seconds-57°C, 30 seconds-72°C) and a final elongation of 5 minutes at 72°C. The 265 bp amplicon was subjected to overnight digestion at 37°C using the HinfI restriction enzyme, 2 units in 10 μl of amplification product. The identification of restriction products was performed through horizontal electrophoresis in ethidium bromide stained 2% agarose gels. The interpretation was made based on the length of restriction fragments as follows: homozygous genotype for the common 677C allele: 265 bp; heterozygous genotype: 265 bp , 171 bp and 94 bp; homozygous genotype for the variant 677T allele: 171 bp and 94 bp.

The genotypes were centralised in an Excel database and the statistical analysis was performed using Graph-Pad InStat 3.10 and SPSS 16.0 for Windows. Allele and genotype frequencies were compared using the χ² test. The approximate risk is presented as Odds Ratios with 95% Confidence Intervals. Results were considered statistically significant at p-value < 0.05.

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Tab. 3. Risk analysis for spontaneous abortion (SA) compared to controls

<table>
<thead>
<tr>
<th>Model</th>
<th>Pathology</th>
<th>Odds ratio</th>
<th>p value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All SA cases</td>
<td>1.367</td>
<td>0.219</td>
<td>0.840, 2.221</td>
</tr>
<tr>
<td></td>
<td>Sporadic SA subgroup (&lt; 3 SA)</td>
<td>1.264</td>
<td>0.408</td>
<td>0.731, 2.184</td>
</tr>
<tr>
<td></td>
<td>Recurrent SA subgroup (≥ 3 SA)</td>
<td>1.584</td>
<td>0.230</td>
<td>0.796, 3.150</td>
</tr>
<tr>
<td></td>
<td>All SA cases</td>
<td>1.633</td>
<td>0.247</td>
<td>0.753, 3.540</td>
</tr>
<tr>
<td></td>
<td>Sporadic SA subgroup (&lt; 3 SA)</td>
<td>1.214</td>
<td>0.814</td>
<td>0.488, 3.017</td>
</tr>
<tr>
<td></td>
<td>Recurrent SA subgroup (≥ 3 SA)</td>
<td>2.493</td>
<td>0.06</td>
<td>0.974, 6.379</td>
</tr>
</tbody>
</table>

Results

The allele frequencies and genotype distribution for the MTHFR C677T polymorphism does not significantly differ between cases and controls (data are presented in Tab. 2). The observed genotype frequencies were in Hardy-Weinberg equilibrium (p>0.05).

Association analyses have been conducted for all SA cases as well as for the two case subgroups described earlier compared to controls. The results of the comparative analyses are depicted in Tab. 3. Chi-square test was used to test for two hypothetical risk models: the dominant model (the 677T variant is a risk factor in both homozygous and heterozygous status) and the recessive model (only the homozygous 677TT genotype is considered a risk factor).

These results suggest that the simple presence of the MTHFR 677T allele in the individual genotype does not represent a risk factor for idiopathic SA. The homozygous MTHFR 677TT genotype only in case of recurrent spontaneous abortions seems to increase the susceptibility to disease, with a statistical analysis very close to the significance level (p = 0.06).

The same analysis performed by comparing the recurrent SA subgroup with the sporadic SA subgroup yielded insignificant differences (CT + TT genotypes vs. CC genotype: OR 1.253; 95%CI 0.599, 2.62; p 0.58 and TT genotype vs. CC + CT genotypes: OR 2.054; 95% CI 0.752, 5.607; p 0.186).

Discussion

An earlier case-control study on 10 genes with possible thrombophilic effect and SA risk (Goodman et al., 2006), shows that only 3 genotypes could be significantly correlated with the disorder, among which the homozygous MTHFR 677TT genotype was reported (p<0.04). This could also be declared as risk factor for recurrent SA by Coulam et al. (2008). In another approach, Sotiriadis et al. (2007) did not confirm that the 677T allele represents a risk factor for idiopathic pregnancy loss neither in homozygous nor in heterozygous status. The same study shows that the homozygous 677TT genotype does not follow a different distribution in patients with a history of at least 3 SA compared to patients with less than 3 SA.

In the literature review by Bick and Hoppensteadt (2005), the functional variants of MTHFR are reported next to factor V Leiden, prothrombin G20210A and the 4G/5G polymorphism of the plasminogen activator gene (PAI-1) as common causes for recurrent spontaneous abortions. A systematic review on thrombophilia and pregnancy (Roberts, 2005) based on 79 different studies shows that, upon stratification for thrombotic risk factors, the risk for early spontaneous abortion is significantly enhanced when hyperhomocysteinemia was present (OR 6.25, 95%CI 1.37, 28.42). The Guide of the Italian Society of Gynecology and Obstetrics (on the basis of 10 meta-analyses and reviews) states that there is not sufficient evidence to recommend some thrombophilia associated gene variants, including MTHFR C677T and A1298C polymorphisms, to be investigated in patients with idiopathic SA (Lussana et al., 2009). One study on placental vasculopathies and their correlation with the same MTHFR variants concludes that C677T is not significantly associated with placental vascular pathology and it cannot predict maternal hyperhomocysteinemia (Klai et al., 2011). However, the MTHFR 677TT genotype is identified as a high genetic risk factor for recurrent SA, together with the homozygous 5G/5G polymorphism of the PAI-1 gene in a Turkish population (Yenicesu et al., 2009). In agreement with this and also with the present study's findings, the homozygous MTHFR 677TT genotype was found with an increased frequency in recurrent SA patients in a Serbian population (Mitic et al., 2010).

Conclusions

The present study represents the first association approach on MTHFR C677T and idiopathic SA, recurrent or sporadic, in the Romanian population. The present study has some limitations such as the relatively small number of recurrent SA cases or the impossibility to determine maternal plasma levels of homocysteine and folate. However, the here presented data are concordant with other literature reports, which indicate that the homozygous MTHFR 677TT genotype is a possible risk factor for...
recurrent SA, though this is not observed in the case of sporadic SA patients. As previous literature suggests, this association is still subject of debate, probably due to other numerous genetic or environmental factors that could influence the etiopathogenesis of SA. Nevertheless, the present study could represent a first step in the future analysis of other conditions related to this pathology in Romanians. Moreover, this study provides supportive information regarding the genetic epidemiology of certain gene variants in the Romanian population.

Acknowledgements

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References


Royal College of Obstetricians and Gynaecologists (2003).