The role of 401A>G polymorphism of methylenetetrahydrofolate dehydrogenase gene (MTHFD1) in fetal hypotrophy

Rola polymorfizmu 401G>A genu dehydrogenazy metylentetrahydrofolianowej (MTHFD1) w hipotrofii płodu

Anna Lorenc¹, Agnieszka Seremak-Mrozikiewicz¹,², Magdalena Barlik¹,², Hubert Wolski¹,³, Krzysztof Drews¹,²

¹ Division of Perinatology and Women’s Diseases, Poznan University of Medical Sciences, Poland
² Laboratory of Molecular Biology in Division of Perinatology and Women’s Diseases, Poznan University of Medical Sciences, Poland
³ Division of Gynecology and Obstetrics Podhale Multidisciplinary Hospital in Nowy Targ, Poland

Abstract

Introduction: Important role is attributed to genetic polymorphisms influencing enzymatic activity in folate metabolism. These inherited genetic variants may influence fetal growth and fetal hypotrophy development. The aim of the study was to investigate the connection of 401A>G polymorphism of methylenetetrahydrofolate dehydrogenase gene (MTHFD1) with increased risk of fetal hypotrophy.

Material and methods: To the study group 120 women who delivered children with fetal hypotrophy and to the control group 120 healthy women were enrolled. Study group was divided into subgroups according to gestational age at delivery (52 patients <37 weeks, 68 patients ≥37 weeks) and to the neonatal weight (31 mothers of newborns with birth weight <1500 g, 89 mothers of newborns with birth weight ≥1500 g). The genetic analysis was performed with the use of PCR/RFLP method.

Results: We observed statistically higher occurrence of mutated 401A allele in hypotrophy group (401A: 27,1 vs. 18,8%, OR=1,61, p=0,02). At mothers who delivered hypotrophic children weighted more than 1500 g the presence of 401A allele was higher (28,7 vs. 18,8%, OR=1,74, p=0,01). Additionally in mothers who delivered hypotrophic children before 37 gestational week statistically higher frequency of 401A allele has been noted (31,7 vs. 18,8%, OR=2,01, p=0,007).

Corresponding author:
Magdalena Barlik
Division of Perinatology and Women’s Diseases, Poznan University of Medical Sciences
ul. Polna 33, 60-535 Poznan, Poland
tel./fax: 618474651
e-mail: magda.barlik@op.pl
Introduction

Fetal hypotrophy is a disease of complex etiology and limited possibilities of treatment, which makes this condition one of the most important issues in perinatal and neonatal medicine. It could be the reason of impaired fetus development and lead to many complications of perinatal period [1, 2]. According to the current literature about 10% of perinatal mortality is a consequence of intrauterine growth restriction. Moreover 52% of stillbirths are correlated with fetal hypotrophy [3, 4]. It is thought that hypotrophy concerns about 4-7% of the newborns in well developed countries and about 15-20% of the newborns in developing countries [5].

According to the above facts intensive research is focused not only to ensure proper perinatal care, but also on the prevention methods of fetal hypotrophy. Nowadays attention is paid on the disturbances in folate metabolism and its connection with etiology of fetal hypotrophy. Impairment of the folate metabolism and inhibition of nucleic acids synthesis could lead to limitation of cell proliferation and differentiation. In addition hyperhomocysteinemia could negatively influence the embryogenesis and implantation. Finally, genetically conditioned changes in the folate metabolism could also lead to fetal hypotrophy [6, 7].

Recent studies revealed that some genetic polymorphisms which influence the enzymes activity involved in folate metabolism could change homocysteine metabolism, nucleic acids synthesis and DNA methylation. One of the most commonly indicated genetic variants involved in these processes are polymorphisms of MTHFR gene (MTHFR 677C>T, 1298A>C). It seems that also other enzymes of folate metabolism could play an important role in the regulation of homocysteine and folate concentration in serum and erythrocytes. These are, for example, methionine synthase, methionine synthase reductase and methylenetetrahydrofolate dehydrogenase (MTHFD1) [8].

Methylenetetrahydrofolate dehydrogenase is a multifunctional enzyme depended on NADP. It is localized in cell matrix where acts in conversion of tetrahydrofolic acid to its derivatives. Some of these derivatives are crucial for cell cycle where they play role as a cofactors of nucleic acids synthesis [9]. (Picture 1).

Hum et al. revealed that MTHFD1 enzyme is composed of two identical units (100 kD each) and has two functional domains. The aminopeptidic end acts as dehydrogenase and cyclohydrolase with NADP as a cofactor. The carboxypeptidic end acts as formyltetrahydrofolate synthase [10]. According to Barlowe et al. formyltetrahydrofolate synthase participates in

Conclusions: Our results indicated that mutated 401A allele of MTHFD1 gene is essential risk factor of fetal hypotrophy in population of Polish women. Appropriate folate supplementation could be particularly essential in women carriers the genetic polymorphism influencing the folate metabolism.

Key words: fetal hypotrophy / folate metabolism / genetic polymorphism / methyleneterahydrofolate dehydrogenase /
nucleic acids synthesis as an element of enzymatic complex [11]. MTHFD1 gene is located on 14 chromosome (14q24). It was shown that single nucleotide polymorphisms in the MTHFD1 gene are correlated to enzyme activity and in consequence impaired concentrations of folates and homocysteine in families with congenital heart defects [12]. Wang et al. suggested that some genetic variants of MTHFD1 may increase the risk of gastric cancer [13]. Moreover, there was suggested a correlation of MTHFD1 gene with neural tube defects, fetal loss in the second trimester of pregnancy and preterm placental abruption [9, 14].

The aim of the study was to evaluate the correlation of 401G>A MTHFD1 gene polymorphism with the risk of fetal hydropathy.

Material and methods

The study group consisted of 120 women who delivered hydropotrophic newborns (weight <2SD or <10 percentile of expected perinatal weight for gestational age). Mean age of the patients from the study group was 29.3±5.8 years, mean gestational age at delivery 35.0±3.2 gestational week (g.w.). To the control group 120 healthy pregnant women (67 nulliparous and 53 multiparous) who delivered newborn with normal weight (mean weight of the newborns 3490.3±351.3 g) were enrolled. For analysis purposes the study group was divided twice: according to the perinatal weight of the newborn (89 patients who delivered newborns with perinatal weight ≥1500 g, 31 patients who delivered newborns with perinatal weight <1500 g) and according to the gestational age at delivery (68 patients ≥37 g.w., 52 patients <37 g.w.). The exclusion criteria from study and control groups were as follow: systemic internal diseases, inherited defects, genetic syndromes, and multiple pregnancies. All patients were Caucasian of Polish origin. The women were informed about the goal of the study and given their written permission.

The study was approved by Bioethical Committee in Poznan University of Medical Sciences (423/11).

Genomic DNA was extracted from blood leucocytes using QIAamp DNA Blood Mini Kit (QIAGEN Inc., Germany) procedure. Genetic analysis of the frequency of genotypes and alleles of 401G>A MTHFD1 gene polymorphism was performed by polymerase chain reaction and restriction fragments length polymorphism (PCR/RFLP). For amplification following starters were used: 5’TGC ATG CTT TCA TTT ATA ATA TGT TT-3’ and 5’-AAT GAA ACA GTC ATT GAG GTC AC-3’. PCR products were hydrolysed with restriction enzyme BsmAI (Alw261) (Fermentas, Lithuania). Analysis of digested fragments was conducted with 2% agarose gel by electrophoresis. The 401GG genotype was identified at the presence of 130, 42 bp long bands, heterozygous 401GA genotype in presence of 172, 130, 42 bp bands. The homozygous mutated 401AA genotype was recognized in presence of 172 bp band.

Statistical analysis was performed by SPSS 17.0. PL for Windows. As a statistically significant we have considered p value lower than 0.05. Frequencies of genotypes were compared by ANOVA test. Expected genotype frequencies were calculated from allele frequencies applying Hardy-Weinberg equilibrium.

Results

Mean gestational age at delivery was statistically lower in the study group in comparison to the control group (35.0±3.2 vs. 39.2±1.3 g.w., p<0.0001). In the study group cesarean sections were statistically more frequent than in the control group (54.2 vs. 14.2%, p<0.0001). In the control group 80.8% of deliveries were spontaneous and in the study group only 43.3% (p<0.0001).

In the whole group of women with hydropotrophic newborns 401GA genotype and mutated 401AA genotype were more frequent in comparison to the control group, despite the lack of statistical significance (401GA: 39.2 vs. 30.8%, OR=1,44, ns; 401AA: 7,5 vs. 3.4%, OR=2,35, ns).

Picture 1. Reaction catalysed by MTHFD1 and its role in DNA synthesis and homocysteine metabolism (according to Brod yet al., 2002).
Table I. The frequency of alleles and genotypes of 401G>A polymorphism of MTHFD1 gene in mothers of newborns in hypotrophy group and control group.

<table>
<thead>
<tr>
<th>MTHFD1 401G&gt;A</th>
<th>Study group (n=120)</th>
<th>Control Group (n=120)</th>
<th>OR</th>
<th>95%CL</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Observed value n (%)</td>
<td>Expected value (%)</td>
<td>Observed value n (%)</td>
<td>Expected value (%)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>64 (53,3)</td>
<td>53,2</td>
<td>79 (65,8)</td>
<td>66,0</td>
<td>0,59</td>
</tr>
<tr>
<td>GA</td>
<td>47 (39,2)</td>
<td>39,5</td>
<td>37 (30,8)</td>
<td>30,5</td>
<td>1,44</td>
</tr>
<tr>
<td>AA</td>
<td>9 (7,5)</td>
<td>7,3</td>
<td>4 (3,4)</td>
<td>3,5</td>
<td>2,35</td>
</tr>
<tr>
<td>Total</td>
<td>120 (100,0)</td>
<td>100,0</td>
<td>120 (100,0)</td>
<td>100,0</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>175 (72,9)</td>
<td></td>
<td>195 (81,2)</td>
<td></td>
<td>0,62</td>
</tr>
<tr>
<td>A</td>
<td>65 (27,1)</td>
<td></td>
<td>45 (18,8)</td>
<td></td>
<td>1,61</td>
</tr>
<tr>
<td>Total</td>
<td>240 (100,0)</td>
<td></td>
<td>240 (100,0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p=statistically significant

Table II. The frequency of alleles and genotypes of 401G>A polymorphism of MTHFD1 gene in mothers of newborns in hypotrophy group divided according to neonatal weight ≥1500 g, <1500 g and control group.

<table>
<thead>
<tr>
<th>MTHFD1 401G&gt;A</th>
<th>Study group (n=120)</th>
<th>Newborns ≥1500 g (n=89)</th>
<th>Newborns &lt;1500 g (n=31)</th>
<th>Control group (n=120)</th>
<th>Observed value n (%)</th>
<th>OR (p)*</th>
<th>Observed value n (%)</th>
<th>OR (p)**</th>
<th>Observed value n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Observed value n (%)</td>
<td>OR (p)*</td>
<td>Observed value n (%)</td>
<td>OR (p)**</td>
<td>Observed value n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>46 (51,7)</td>
<td>0,55 (0,03)*</td>
<td>18 (58,1)</td>
<td>0,71 (0,27)</td>
<td>79 (65,8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>35 (39,3)</td>
<td>1,45 (0,13)</td>
<td>12 (38,7)</td>
<td>1,41 (0,26)</td>
<td>37 (30,8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>8 (9,0)</td>
<td>2,86 (0,07)</td>
<td>1 (3,2)</td>
<td>0,97 (0,72)</td>
<td>4 (3,4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>89 (100,0)</td>
<td></td>
<td>31 (100,0)</td>
<td></td>
<td>120 (100,0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>127 (71,3)</td>
<td>0,57 (0,01)*</td>
<td>48 (77,4)</td>
<td>0,79 (0,30)</td>
<td>195 (81,2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>51 (28,7)</td>
<td>1,74 (0,01)*</td>
<td>14 (22,6)</td>
<td>1,26 (0,30)</td>
<td>45 (18,8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>178 (100,0)</td>
<td></td>
<td>62 (100,0)</td>
<td></td>
<td>240 (100,0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*, ** p=statistically significant

Table III. The frequency of alleles and genotypes of 401G>A polymorphism of MTHFD1 gene in mothers of newborns in hypotrophy group divided according to gestational age at delivery ≥37 g.w., <37 g.w. and control group.

<table>
<thead>
<tr>
<th>MTHFD1 401G&gt;A</th>
<th>Study group (n=120)</th>
<th>Delivery ≥37 g.w. (n=68)</th>
<th>Delivery &lt;37 g.w. (n=52)</th>
<th>Control group (n=120)</th>
<th>Observed value n (%)</th>
<th>OR (p)*</th>
<th>Observed value n (%)</th>
<th>OR (p)**</th>
<th>Observed value n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Observed value n (%)</td>
<td>OR (p)*</td>
<td>Observed value n (%)</td>
<td>OR (p)**</td>
<td>Observed value n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>41 (60,3)</td>
<td>0,79 (0,27)</td>
<td>23 (44,2)</td>
<td>0,41 (0,007)**</td>
<td>79 (65,8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>22 (32,3)</td>
<td>1,07 (0,47)</td>
<td>25 (48,1)</td>
<td>2,08 (0,024)**</td>
<td>37 (30,8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>5 (7,4)</td>
<td>2,30 (0,18)</td>
<td>4 (7,7)</td>
<td>2,42 (0,19)</td>
<td>4 (3,3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>68 (100,0)</td>
<td></td>
<td>52 (100,0)</td>
<td></td>
<td>120 (100,0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>104 (76,5)</td>
<td>0,75 (0,17)</td>
<td>71 (68,3)</td>
<td>0,49 (0,007)**</td>
<td>195 (81,3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>32 (23,5)</td>
<td>1,33 (0,17)</td>
<td>33 (31,7)</td>
<td>2,01 (0,007)**</td>
<td>45 (18,8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>136 (100,0)</td>
<td></td>
<td>104 (100,0)</td>
<td></td>
<td>240 (100,0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*, ** p=statistically significant
In the control group statistically significant over-representation of 401G genotype was observed (53.3% vs. 65.8% in the control group, OR=0.59, p=0.03). The frequency of mutated 401A allele was statistically higher in the study group and the frequency of 401G allele was statistically higher in the control group (401A: 27.1% vs. 18.8% in the control group, OR=1.61, p=0.02; 401G: 72.9% vs. 81.2% in the control group, OR=0.62, p=0.02), (Table I).

In the subgroup of women with newborns’ weight ≥1500 g the frequency of mutated 401AIA genotype was higher (9.0% vs. 3.4%, OR=2.86, p=0.07). Also heterozygous 401G4A genotype was more frequent in this subgroup (39.3 vs. 30.8%, OR=1.45, ns). The frequency of mutated 401A allele was statistically higher in the subgroup of women with newborns’ weight ≥1500 g (28.7 vs. 18.8%, OR=1.74, p=0.01). In the subgroup of women with newborns’ weight <1500 g there were no statistically significant differences, but interestingly the overrepresentation of heterozygous 401G4A genotype (38.7 vs. 30.8, OR=1.41, ns) such as mutated 401A allele (22.6 vs. 18.8%, OR=1.26, ns) has been also observed. (Table II).

In the subgroup of women who delivered <37 g.w. statistically lower frequency of 401GG genotype was observed (44.2% vs. 65.8% in the control group, OR=0.41, p=0.007). Moreover, the frequency of 401GIA genotype was statistically higher in this subgroup in comparison to the control group (48.1 vs. 30.8%, OR=2.08, p=0.024). Similar observations concerned alleles of 401G>A MTHFD1 polymorphism. The 401G allele was less frequent in the subgroup <37 g.w. (68.3 vs. 81.3%, OR=0.49, p=0.007) and mutated 401A allele was more frequent in the group <37 g.w. (31.7 vs. 18.8%, OR=2.01, p=0.007).

In the subgroup of women who delivered ≥37 g.w. the frequency of mutated 401AIAA genotype was higher than in the control group (7.4 vs. 3.3%, OR=2.30, ns). The frequency of mutated 401A allele also was higher in women delivered ≥37 g.w. (23.5 vs. 18.8%, OR=1.33, ns.) (Table III).

Discussion
There was shown a lot of risk factors influencing the fetal hypotrophy development. Each fetus has a genetically determined potential growth that is inherited from parents. This could be modulated by some fetal diseases, mothers health and placental function. If factors in this delicate balance do not act synergically, the fetal growth could be restricted and fetal hypotrophy may develop. Unfortunately the treatment possibilities in fetal hypotrophy are very limited, standard procedure involves mainly fetal observation with ultrasonography and cardiotocography [15, 16, 17].

Currently there are also some researches concerning prophylaxis of this condition with using L-arginine, acetylsalicylic acid or heparin. There are encouraging evidences for efficacy of that kind of prophylaxis methods [18].

On the other hand some studies showed how important in fetal hypotrophy could be prophylaxis by proper folate supplementation. Folate requirement significantly increases during pregnancy. Too low folate supplementation leads to decrease of folate concentration in plasma and erythrocytes [2]. Baker et al. revealed correlation of insufficient folate supplementation (<187 μg/day) and the risk of fetal hypotrophy [19].

Some studies showed that high folate concentration in third trimester are correlated with proper birth weight and lower risk of hypotrophy [20]. The others authors investigated connection between folate supplementation and hypotrophy and indicated statistically significant correlation between serum folate concentration and newborn’s weight [21]. It was also observed influence of folate supplementation on the decreased risk of fetal hypotrophy [22]. The study of Leeda et al. shows the importance of proper folate supplementation in pregnant women with a positive history of hypotrophy. Moreover, the authors suggest that higher folate concentration in erythrocytes correlated with lower risk of fetal hypotrophy [23].

The important issue connecting folate metabolism and hypotrophy development are the genetic polymorphisms influencing enzymes activity in folate cycle. To our knowledge this is the first study that evaluate the correlation of 401G>A MTHFD1 gene polymorphism with fetal hypotrophy. In present study obtained results showed an significant association of 401G>A polymorphism of MTHFD1 gene in mothers with the risk of hypotrophy.

In the whole group of women with fetal hypotrophy overrepresentation of mutated 401A allele was observed (401A: OR 1,16, p=0,02). The distribution connected with birth weight (≤1500 g and >1500 g) was adopted because the 1500 g fetal weight is the limit of very low birth weight connected with increased percent of perinatal complications. The other one criteria was the 37 gestational week of delivery which is related to term pregnancy.

In our analysis it was shown that the frequency of mutated 401A allele were statistically higher in the group of women delivered <37 g.w. (401A: OR=2.01, p=0.007). The frequency of mutated 401A allele also was statistically higher in the subgroup of women delivered newborns with perinatal weight ≥1500 g (OR=1,74, p=0.01). Moreover mutated 401A allele was overrepresented in the group of women delivered newborns weighted ≥1500 g (OR=1,26, ns). The analysis showed that independently if this group was divided according to birth weight and gestational week of delivery always mutated 401A allele remained a risk factor for fetal hypotrophy.

Fetal hypotrophy is a significant obstetrical problem with serious clinical consequences. According to lack of intrauterine procedures of fetal hypotrophy treatment, early diagnosis, obstetrical care and prevention methods seem to be the most important [18, 24]. Many studies show that crucial part of hypotrophy prevention may be proper folate supplementation. Genetic studies of hypotrophy could be very helpful to identify a group of women with impaired enzyme activity in whom prevention may have expected benefits.

Conclusions
1. Our results indicated that mutated 401A allele of MTHFD1 gene is essential risk factor of fetal hypotrophy in population of Polish women.
2. Appropriate folate supplementation could be particularly essential in women carriers the genetic polymorphism influencing the folate metabolism.
Oświadczenie autorów

1. Anna Lorenc – autor koncepcji i założeń pracy, przygotowanie manuskryptu i pisemnościwa – autor zgłaszający i odpowiedzialny za manuskrypt
3. Magdalena Barlik – współautor tekstu pracy i protokołu, korekta i aktualizacja literatury.
4. Hubert Wolski – analiza i interpretacja wyników, przygotowanie, korekta i akceptacja ostatecznego kształtu manuskryptu.
5. Krzysztof Drews – ostateczna weryfikacja i akceptacja manuskryptu.

Zródło finansowania:
Praca nie była finansowana przez żadną instytucję naukowo-badawczą, autorzy nie otrzymali żadnego grantu.

Konflikt interesów:
Autorzy nie zgłaszają konfliktu interesów oraz nie otrzymali żadnego wynagrodzenia związku z powstawaniem pracy.

References