Gene Reports 10 (2018) 116–122

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

A family based triad study evaluating the role of MTHFR gene polymorphisms in spontaneous abortions

M. Vidyadhari[a], M. Sujatha[a], P. Krupa[b], Pratibha Nallari[a], A. Venkateshwari[a,*]

[a] Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet, Hyderabad, India
[b] Government Modern Maternity Hospital, Petlaburz, Hyderabad, India

ARTICLE INFO

Keywords:
Spontaneous abortion
Family based triad study
Polymorphism
Polymerase chain reaction
Folic acid
Aborted fetuses
Haplotype

ABSTRACT

Objective: To explore the combined association of MTHFR gene polymorphisms (C677T, A1298C) with spontaneous abortions in a triad group from Telangana State of South Indian population.
Materials: A total of 80 case families with spontaneous abortions and 100 control families with medically terminated pregnancies were considered. Fetal tissue with < 20 weeks of gestational age and peripheral blood from their respective parents were collected.
Methods: DNA was isolated by phenol-chloroform method from both tissue and peripheral blood samples and then genotyped using Amplification Refractory Mutation System–Polymerase Chain Reaction followed by agarose gel electrophoresis. Genotype and allelic frequencies of MTHFR polymorphisms were determined by Odds Ratio with 95% Confidence Intervals and level of significance was considered at p value < 0.05. The mRNA secondary structures were predicted and the transcription factor binding sites were determined.
Results: Statistically significant association was observed with 677C/T polymorphism in case fetal (p = 0.006), maternal (p < 0.001) and paternal (p = 0.03) groups compared to their respective control groups. A significant difference in the genotypic distribution of 1298 A/C polymorphism was observed only in the maternal group (p = 0.005) compared to controls. The combined frequencies of 677TT/1298AC genotypes revealed a significant association with an increased risk in all the case triad groups compared to their respective controls. The in silico analysis revealed the structural change and transcription factor binding site deviations in the variant allele compared to wild type allele in the mRNA regulation of MTHFR protein.
Conclusion: The present study supports the major role of MTHFR gene polymorphisms in the etiology of spontaneous abortions.

1. Introduction

Spontaneous abortion is the most common reproductive complication leading to the natural death of the fetus before 20 weeks of gestational age in mother's womb itself. It occurs in the first trimester of pregnancy that terminates spontaneously before fetal viability (Coulam et al., 1997). Among all clinically recognized pregnancies, 10%–15% of them were due to spontaneous abortions (Wilcox et al., 1988). The most common cause of spontaneous abortions is due to the genetic defects than anatomical, infectious and immunological factors (Zolghadri et al., 2011). Folic acid plays a major role in the growth and development of the embryo during pregnancy (Robertson et al., 2006; Bokarewa et al., 1996). Low folic acid may act as an embryotoxin causing various birth defects. An increased folic acid intake is recommended during prenatal period as there is a possibility of folic acid deficiency naturally (Cai et al., 2010).Thus, pregnant women with low concentrations of folic acid had an increased risk of spontaneous miscarriages than women with required concentrations of folic acid (Forges et al., 2007). Hence, sufficient folate supplementation during early pregnancy may be beneficial which reduce its adverse effects in the embryogenesis and placental vasculature. MTHFR (methylenetetrahydrofolate reductase) is an important folate metabolizing enzyme that converts inactive folic acid into its active form, 5-methyltetrahydrofolate which is immediately absorbed rather than the inactive form (Mukhopadhyay et al., 2009) and is located on chromosome 1 at 1p36.3 with 11exons (Gilbody et al., 2007; Mtiraoui et al., 2006). Deficiency of MTHFR, leads to halting of active folate and associated with increased pregnancy related complications. Two common polymorphisms, C677T and A1298C among

Abbreviations: MTHFR, methylenetetrahydrofolate reductase; ARMS-PCR, Amplification Refractory Mutation System–Polymerase Chain Reaction method; OR, odds ratios; CI, confidence intervals; MFE, minimum free energy; kcal/mol, kilocalorie/mole; mRNA, messenger ribonucleic acid

* Corresponding author.
E-mail address: venkateshwari@yahoo.com (A. Venkateshwari).

https://doi.org/10.1016/j.genrep.2017.12.001
Received 26 August 2017; Received in revised form 18 November 2017; Accepted 1 December 2017
Available online 05 December 2017
2452-0144/ © 2017 Published by Elsevier Inc.

several allelic variants of MTHFR gene were considered to cause 20%–30% decrease in the enzyme activity leading to hyperhomocystinemia with decrease in folic acid intake. It further leads to alteration in feto-maternal circulation, fetal viability and the placentation process during the development of embryo. Thus the MTHFR gene and its encoded product is interlinked with the complications in embryonic development during pregnancy leading to increased risk of spontaneous miscarriages (Trabetti, 2008). A 677C/T transition is a missense mutation located in the coding region of exon 4, that converts an alanine to a valine at codon 222 resulting in decreased enzymatic activity (Unfried et al., 2002). A1298C transversion is a point mutation in exon 7, substituted by a glutamate to alanine at codon 429 leading to a reduced enzyme activity, but to the less extent than 677T allele (Ananth et al., 2007).

Hence, the present study is aimed to evaluate the distribution of MTHFR polymorphisms (677C > T and 1298 A > C) in fetal, maternal and paternal samples and occurrence of spontaneous abortions in Telangana state of South Indian population.

2. Materials and methods

2.1. Sample collection

The samples of case triad group and control subjects for the present study were collected from the Department of Obstetrics and Gynecology, Government Maternity Hospital, Hyderabad, Telangana state during Feb, 2013 to April 2017. A total of 80 spontaneously aborted fetal tissues with their parents (80 + 160 = 240) and 100 medically terminated normal fetal tissues with their parents (100 + 200 = 300) were considered in the study. Medically terminated fetuses are considered as control fetuses, which are considered normal. Aborted fetal tissues (50 mg) were collected from the mothers of spontaneous abortions and medically terminated pregnancies. A 5 ml of peripheral blood was collected in EDTA vaccutainers from all the case and control couples for the genotyping of MTHFR gene polymorphisms. Demographic characteristics such as maternal age, paternal age, gestational duration, number of miscarriages, consanguinity, etc. was obtained with the help of a standard proforma. Detailed clinical information from all the subjects were recorded before sample collection along with an informed consent. The investigation of toxoplosmosis, hepatitis B and C, cytomegalovirus, rubella, HIV, protein C, protein S, prolactin dosage, thyroid hormone levels, glucose levels, antiphospholipid antibodies were also investigated throughout their gestational period. The hysteroscopy, hysterosalpingography, serial ultrasound scan to identify polycystic ovaries and transvaginal ultrasound was carried out to confirm the spontaneous abortion. The present study was approved by the ethical committee of the Institute of Genetics, Osmania University, Hyderabad.

2.2. The inclusion criteria

The case couples with atleast two consecutive spontaneous abortions before 20th week of gestational age without any normal pregnancy in the previous history were included. The control group of same ethnic population with at least two normal pregnancies and absence of previous history of abortions were considered. Lack of infertility complications with no reproductive problems and known medical illnesses were included as controls in the present study. All the fetuses within 20 weeks of gestational age were considered.

2.3. The exclusion criteria

The subjects with risk factors like chronic infections, thrombosis, autoimmune diseases, endocrinological disorders, or congenital anomalies were excluded from the present study. Participants under continuous usage of oral contraceptives and any other medications during the course of pregnancy were not included in the study.

2.4. Determination of MTHFR gene polymorphisms

Genomic DNA was isolated from fetal tissues and peripheral blood by modified phenol chloroform method (Unfried et al., 2002). The DNA was then genotyped by Amplification Refractory Mutation System–-Polymerase Chain Reaction method (ARMS-PCR) for the detection of MTHFR (677C/T, 1298 A/C) gene polymorphisms using sequence specific primers.

2.4.1. MTHFR 677C/T genotype

The primers used were 5′-TGC TGT TGG AAG GTG CAA GAT-3′ or 5′-GCG TGA TGA TGA AAT CGG-3′ with 5′-GCG TGA TGA TGA AAT CGA-3′ to detect the band patterns at 226 bp. PCR was carried out on 20 μl volume, in an eppendorf thermal cycler (eppendorf, Germany). First denaturation step (96 °C, 2 min) was followed by 10 cycles of denaturation (96 °C, 15 s) and annealing/extension (65 °C, 60 s), and a final 20 cycles of denaturation (96 °C, 10 s), annealing (61 °C, 50 s), and extension (72 °C, 30 s) (Jeddi-Tehrani et al., 2011). The PCR products were electrophorosed on 2% agarose gel and stained with ethidium bromide.

2.4.2. MTHFR 1298 A/C genotype

The primers used were 5′-CCTTTGGGGAGCTGAAGGACTACTAC-3′ or 5′-CAAAGGACTTCAAAGACAGTC-3′ with 5′-GGTAAAGAACAAAGA CTTCAAAGACACTGTG-3′ to detect two bands at 120 bp and 127 bp. The reaction conditions were set as follows: initial denaturation at 95 °C for 10 min, followed by 20 cycles of amplification at 92 °C for 15 s and 60 °C 1 min, an additional 30 cycles of amplification at 89 °C for 15 s and 60 °C for 1.5 min, and a final extension at 72 °C for 10 min. PCR-products were visualized by 2% agarose gel electrophoresis using ethidium bromide (Hoppe et al., 2003). 10% of the samples were taken randomly and regenotyped for validation. The findings revealed 100% concordance with the similar results.

2.5. Statistical analysis

Allele frequencies and genotypic distribution in case group (spontaneously aborted fetuses and their parents) and control group (medically terminated fetuses and their parents) were analyzed by Open Epi two x two table. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated to measure the strength of association between MTHFR gene polymorphisms (677C/T, 1298 A/C) and spontaneous abortions. The p-values were determined by two-tailed Fisher's exact test. The p value < 0.05 was regarded as statistically significant.

2.6. In silico analysis

The original DNA sequences of the MTHFR gene (with AC: NG_013351) polymorphisms were considered from National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/nucleotide). The evaluation of the effect of C677T transition and A1298C transversion on the mRNA structures of the protein was predicted using the RNAfold Web Server (http://rna.tbi.univie.ac.at/RNAfold). The prediction of transcription factor binding sites involved in mRNA regulation of MTHFR protein were determined by AliBaba2.1 database using TRANSFAC 4.0 sites (http://gene-regulation.com/pub/programs/alibaba2/index.html).

3. Results

A total of 300 control samples and 240 case samples were considered for the study. Demographic characteristics from 100 medically terminated couples and 80 spontaneously aborted couples were shown in the Table 1. The analysis of the demographic characteristics showed

Download English Version:

https://daneshyari.com/en/article/8646261

Download Persian Version:

https://daneshyari.com/article/8646261

Daneshyari.com