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Hereditary thrombophilia genetic variants in recurrent pregnancy loss

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Abstract

Background The relationship between thrombophilia genes and recurrent pregnancy loss has been discussed. The aim of this study was to investigate the association between of MTHFR C677T, A1298C, F2G20210A, and F5 G1691A genetic variants among Iranian women with recurrent miscarriage.

Methods A total of 245 women with two or more recurrent pregnancy loss, with mean age years were enrolled in the study. To compare genotypes, we have selected 250 healthy women without history of miscarriage as control group. Genomic DNA of participants was evaluated using polymerase chain reaction followed by Sanger sequencing to determine the genotype frequency.

Results The mean age were 32.16 ± (21–42) and 31.81 ± (19–40) for case and control groups respectively. MTHFR C677T and A1298C mutant alleles were found to be significantly more prevalent in patients than control. However, F2G20210A and F5 G1691A genetic variants showed no significance.

Conclusion The allele frequencies for the assessed genotypes in this study are consistent with the data obtained for other countries. We observed significant susceptible effects of MTHFR C677T, and A1298C among participants. According to the relatively high prevalence of these variants, we recommend genetic testing for women with RPL before therapeutic decisions.

Keywords Thrombophilia · Variants · MTHFR · Recurrent pregnancy loss

Introduction

Recurrent pregnancy loss (RPL) is well defined as two or more spontaneous miscarriage which affects approximately 5% of women in reproductive age [1, 2]. There are many factors which play role in etiology of RPL and despite inclusive endocrine, anatomic, immunologic, and chromosomal evaluation, 30–40% of cases of RPL remain unsolved [3]. In recent times, it has been suggested that thrombophilia factors are a possible cause of RPL [4, 5] which are defined as predisposition for thrombosis. Thrombophilia may be inherited or acquired. About 40% of cases presenting with

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thrombosis are inherited. Hereditary thrombophilia has been shown to be a risk factor for reproductive disorders including infertility [6, 7], recurrent pregnancy loss [8-10], and obstetrical complications [11, 12]. While the association of specific genetic variants with the various types of reproductive problems has been controversial, a number of reports have demonstrated that the risk of reproductive disorders increases with increasing numbers of genetic risk factors [13, 14]. The 677C > T mutation (rs1801133) of the MTHFR gene leads to a defective methylene tetrahydrofolate reductase (MTHFR) enzyme that has only 50% of the normal activity and is a common cause of elevated levels of homocysteine, previously identified as a risk factor for VTE. The American College of Obstetricians and Gynecologists recommends against screening for MTHFR variants to determine the presence of thrombophilia in pregnant women [15]. On the other hand, the co-inheritance of the alleles linked to thrombophilia, such as the F5 1691A, F2 20210A, and MTHFR 677 T, may increase the risk for PL. The F2 20210G > A (rs1799963) mutation results in increased prothrombin levels and is considered as another risk factor for VT [10]. The heterozygotes have two-to-fivefold increased risk of thrombosis which may also increase the risk for PL. The prevalence of F2 20210G > A in European Caucasians is from 1 to 8%, and from 1 to 12% in Mediterranean populations. The F5 gene mutation 1691G > A (rs6025) results in an altered variant of factor V, namely Factor V Leiden, which cannot be easily cleaved by activated protein C (aPC). The 1691G > A mutation increases the risk of venous thrombosis (VT) up to 50–100-fold in adult homozygous. Numerous studies indicated that the F5 1691G > A is responsible for 3–42% of PL [16]. Therefore, we aimed to investigate the prevalence and association of MTHFR C > T (rs1801133), MTHFR A > C (rs1801131)F2 G > A (rs1799963), and F5 G > A (rs6025) variants in Iranian women with RPL.

Materials and methods

Patients and samples

A total of 245 with a history of two or more repeated abortions between 2016 and 2018 were conducted at the Next Generation Genetic Policlinic, Mashhad, Iran. These participants were evaluated for hormonal (TSH and Prolactin) and anatomic abnormalities using ultrasonography of the uterine and genital tract previously which have been followed up by an expert gynecologist and medical geneticist based on standard methods [17]. All participants have been assessed for common thrombophilic factors such as protein C and protein S, anti-thrombin, and anti-cardiolipin antibodies (G, M), through the same diagnostic methods before genetic counseling. Two hundred and fifty age- and ethnicity-matched women were also recruited as control group. All control subjects had two or more living children without previous history of RPL. Written informed consent from all the participants were obtained.

Genotyping analysis

Genomic DNA was extracted from peripheral blood leucocytes using the commercially available kit (QIAGEN GmbH, Hilden Germany). Four thrombophilia gene variants including MTHFR (677 C > T and 1298 A > C), F2 and F5 were determined using polymerase chain reaction (PCR) amplification according to optimized protocol followed by Sanger sequencing.

Statistical analysis

Results are presented as mean—standard deviation. Differences in variables were statistically analyzed with the Student's *t* test and Chi-square test, when appropriate. A p value < 0.05 was considered statistically significant. All data analyses were performed using SPSS software (version 16; IBM SPSS, NY, USA), and two-sided p values < 0.05 were considered statistically significant.

Results

The mean age was 32.16 ± (21–42) and 31.81 ± (19–40) for case and control groups, respectively. All sequences have been aligned with the human genome References 38 (GRCh38) and chromatograms were evaluated to find heterozygote and homozygote variants (Fig. 1). All genetic variants in cases and controls were in Hardy–Weinberg equilibrium except for F5 ($p = 0.000$) in the RPL group. Table 1 shows the prevalence of tested variants among study groups. MTHFR C677T and A1298C mutant alleles in RPL cases were significantly higher than control group ($p = 0.000$). However, A > G alleles in both F2 and F5 genes were not significantly different. Statistical analysis of all selected variants using logistic regression showed C > T nucleotide change, will increase the RPL risk as 5.5-fold. Moreover, A > C change leads to increase the risk of RPL as 3.3-fold. Otherwise, G > A nucleotide alteration for both F2 and F5 selected variants have not shown significant risk in our study ($p > 0.05$). Table 2 shows the association of the MTHFRC677T and A1298C with RPL in the study groups.

Discussion

Recurrent pregnancy loss is a challenging condition, both medically and emotionally. While most cases remain unexplained, inherited thrombophilia has recently been implicated as a potential cause [18]. So far, different definitions

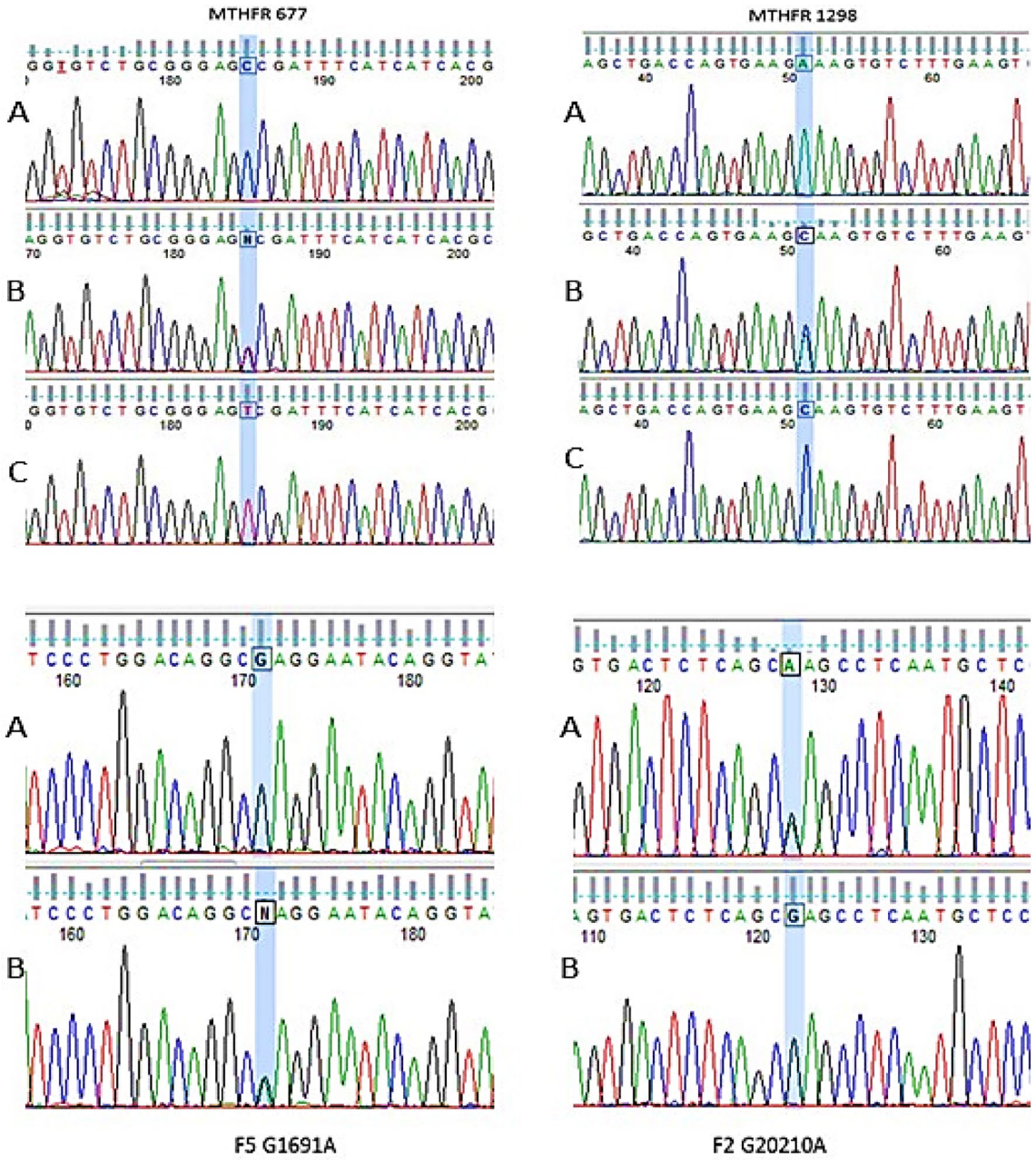

Fig. 1 Chromatogram of all selected variants in MTHFR, F2 and F5 genes. A, B, and C corresponds for wild-type homozygote, heterozygote, and mutant heterozygotes, respectively

have been introduced for RPL. Several authors and societies, such as the WHO, define RPL as three or more abortions before the 20th week of gestation [19, 20]. While other authors and new guidelines have defined it as two or more [17, 21]. However, to which extent these definitions needs to be extended or constricted is less clear, as is shown by different definitions used in different guidelines and different countries. Although we have not found a significant difference according to the number of losses, there is some evidence from one observational study that whether the pregnancy losses are consecutive or not, or two versus three losses is not associated with the risk of pregnancy loss [22].

The aim of the present study was to evaluate the prevalence of MTHFR C > T (rs1801133), MTHFR A > C (rs1801131), F2 G > A (rs1799963), and F5 G > A (rs6025) variants in Iranian women with RPL. To date, several lines of evidence indicate that a mixture of risk factors, including multiple inherited thrombophilia disorders associated with secondary hypercoagulable states, has a particularly strong relationship with an adverse pregnancy outcome [23].

Table 1 Distribution of thrombophilia genetic variants among RM cases and controls

Genotypes	Patients ($n=245$)	Control ($n=250$)	p value
MTHFR C677T			
CC	127 (51.8%)	222 (88%)	0.000
CT	95 (38.8%)	22 (8.8%)	
TT	23 (9.4%)	6 (2.4%)	
C Allele	348 (71.2%)	466 (93.2%)	0.000
T Allele	142 (28.8%)	34 (6.8%)	
MTHFR A1298C			
AA	83 (33.9%)	179 (71.6%)	0.000
AC	129 (52.7%)	57 (22.8%)	
CC	33 (13.4%)	14 (5.6%)	
A Allele	294 (60%)	416 (83.2%)	0.000
C Allele	196 (40%)	84 (16.8%)	
F11 G20210A			
GG	238(97.1%)	246 (98.4%)	0.261
GA	7 (2.9%)	4 (1.6%)	
AA	0 (0%)	0 (0%)	
G Allele	484 (98.8%)	496 (99.2%)	0.351
A Allele	6 (1.2%)	4 (0.8%)	
F5G1691A			
GG	234 (95.5%)	245 (98%)	0.188
GA	9 (3.7%)	5 (2%)	
AA	2 (0.8%)	0 (0%)	
G Allele	476 (97.1)	496 (99.2%)	0.163
A Allele	14 (2.9)	4 (0.8%)	

MTHFR is a main regulator enzyme which is involved in the DNA synthesis and homocysteine methylation cycle. Moreover, several studies showed that it plays an important role in female reproduction as well [24]. Accumulation of homocysteine could be responsible for the production of reactive oxygen species which could results in embryo low cleavage rates, high embryonic fragmentation, and low rates of blastocyst formation [25]. Otherwise, antioxidant properties of folic acid protect cellular membranes, which inhibit lipid peroxidation and DNA damage from free radicals. Reduced enzyme activity could restrict oocyte maturation, ovulation, luteolysis, and follicle atresia [26]. It has been shown that the C677T mutation is associated with a threefold increased risk of miscarriage [27]. The frequency of MTHFR 677 T allele in Bosnians is 37.5% and is higher than those reported for the Czechs, Ukrainians, Norwegians, and Swedes (25.0%, 29.1%, 29.0%, and 25.0%, respectively), as well as for Austrians and the Dutch (each 29.3%) [15]. In this study, the prevalence of heterozygotes and homozygote for the rs1801133 (allele T) was 38.8% and 9.4%, respectively, which there was significantly higher than control group ($p=0.000$). In a study it has been reported that the frequency of homozygous mutations for MTHFR C677T was also increased in women experiencing recurrent pregnancy loss compared with controls [28]. In this study, the frequency of homozygotes women for A1298C was 13.4% which was higher than control group (5.6%) significantly ($p=0.000$). Combination of MTHFR C677T with A1298C in RPL have been reported previously. Isotalo et al. provided the primary evidence that combined MTHFR 677T and 1298C variants may compromised fetal viability. In addition, the role of human MTHFR 677T/1298C and 677TT/ 1298CC genotypes in fetal viability has been explained [29]. The cis-mutation identification is more significant, because it allows more than two mutant alleles to be present in the genome of fetus. If *c* is MTHFR configuration happened, they would result in selection disadvantages because of the expression of severe phenotypes include spontaneous abortion [30]. Moreover, it has been showed that homozygous variants of

Table 2 Genetic Models among RM cases and controls

Genotypes	Patients ($n=245$)	Control ($n=250$)	OR	95% CI	p value
MTHFR C677T					
TT + CT Vs CC (dominant model)			7.36	4.62–11.74	0.000*
TT Vs CT + CC (recessive model)			4.21	1.68–10.53	0.000*
C Allele	348 (71.2%)	466 (93.2%)			
T Allele	142 (28.8%)	34 (6.8%)	5.593	3.752–8.336	0.000*
Codominant model			6.701	2.658–16.891	0.000*
MTHFR A1298C					
CC + AC Vs AA (dominant model)			4.92	3.36–7.20	0.000*
CC Vs AC + AA (recessive model)			2.62	1.36–5.03	0.003*
A Allele	294 (60%)	416 (83.2%)			
C Allele	196 (40%)	84 (16.8%)	3.302	2.456–4.439	0.000*
Codominant model			5.083	2.583–10.006	0.000*

*$p<0.05$ considered as significant

both SNPs (677T/T and 1298C/C) have a significant role in pregnancy complications [31].

The two most common hereditary thrombophilia factors are F5 and F2 variants [32]. F5 is a point substitution (G1691A) altered factor V, which results in resistance to inactivation by protein C and consequence hypercoagulable state with a five-to-tenfold risk of thrombosis in heterozygote and an 80-fold risk in homozygote individuals [33]. Factor V Leiden is responsible for 20–40% of isolated thrombotic events and 40–45% of familial thrombophilia. The prevalence of F5 in the United States is estimated to be between 3 and 7%, with the highest frequency in whites. Many studies have investigated the relationship between F5 and RPL, and the majority found an association, with odds ratios ranging from 0.5 to 18 [9, 33, 34]. In this study, 2.9% and 1.6% of cases and controls were heterozygous which not significantly different ($p=0.261$). We found no homozygous AA variant in any participants which is compatible with the other reported studies. Due to an important role of F2 gene and early detection of any defect in the protein function, the studies indicated that F2 G20210A variant was not found in any RPL cases [34, 35].

Conclusion

With growing awareness about the role of genetic factors influencing hemostasis and pregnancy-related disorders, documentation of thrombotic causes is important because of the potential of performing randomized-controlled clinical trials to determine the effective prevention of thrombotic events in such cases. Moreover, the importance of such study cannot be ignored. Further studies with focus on functionally significant variants in other related genes involved in the coagulation pathway in women with RPL can be helpful to find other risk factors. According to the relatively high prevalence of these variants, we recommend genetic testing for women with RPL before therapeutic decisions.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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