Association between in vitro fertilization outcomes and inherited thrombophilias: a meta-analysis

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Received: 3 February 2016 / Accepted: 28 April 2016 / Published online: 23 May 2016
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Abstract

Purpose The aim of this study was to determine whether in vitro fertilization (IVF) outcomes are associated with inherited thrombophilias.

Methods Several databases including PubMed, Embase, and Cochrane Library were retrieved up to 12 January 2016. The quality of the included studies was assessed by two authors. The associations of the following mutations in inherited thrombophilias and IVF outcomes were explored: factor V Leiden (FVL), prothrombin gene G20210A mutation (PGM), 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T, MTHFR (A1298C) and activated protein C resistance (APCR). The main outcome measures included CPR and implantation rate (IR). The relative risk (RR) and its 95 % confidence interval (CI) were calculated for effect index. Heterogeneity test was evaluated by Chi-square based on Q statistic and I^2 statistics.

Results A total of seven articles published between 2007 and 2015 with the ages of subjects between 30.9 and 36.2 were included. For subgroups analysis of CPR or IR, there were no

significant differences in MTHFR (C377T), MTHFR (A1298C), FVL, PGM, and FVL/PGM mutation were found between the mutation group and control group ($P>0.05$).

Conclusions IVF outcomes are not associated with FVL, PGM, MTHFR (C677T), MTHFR (A1298C), and APCR mutation in inherited thrombophilias.

Keywords Meta-analysis · Inherited thrombophilias · In vitro fertilization

Introduction

Even after transferring morphologically normal embryos, many women undergoing in vitro fertilization (IVF) fail to become pregnant. Success of given IVF cycle is confirmed by implantation. Complex interactions of recurrent pregnancy loss and infertility including inherited thrombophilias could affect implantation [1, 2]. Disagreement exists regarding whether inherited thrombophilias have effects on implantation.

Multiple studies have reported that thrombophilias result in implantation failure [3–5]. Specifically, a small study on inherited thrombophilias revealed that compared with fertile patients who achieved pregnancy by natural conception or patients who succeeded with the first IVF attempt, combinations of two or more of these thrombophilic mutations were more often shown recurrent implantation failure [4]. In addition, a similar study surveyed the role of the coagulation/fibrinolytic system in implantation and found that patients with recurrent implantation failure experienced reduced plasma fibrinolytic potential compared with fertile controls or patients with the first cycle of IVF [6]. Conversely, other investigators have also studied the correlations between thrombophilias and implantation, and have conflicting results [7, 8]. These studies examined the effects of inherited thrombophilias not on factor V Leiden, prothrombin,

Capsule IVF outcomes are not associated with FVL, PGM, MTHFR (C677T), MTHFR (A1298C), and APCR mutation in inherited thrombophilias.

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Highlights 1. A total of seven articles published between 2007 and 2015 were included
2. No significant difference in CPR or IR was observed between the studies
3. No significant difference in IR was observed between the studies

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and methylenetetrahydrofolate reductase, and found that there were no differences between fertile controls who conceived naturally and infertility patients who experiencing failed IVF.

There is clearly a disagreement regarding the role of inherited thrombophilias in implantation failure and IVF outcomes. It is difficult for a meta-analysis to conclude conclusive results for these discordant findings between retrospective studies and small prospective studies [9]. Therefore, our objective was to resolve this disagreement through more patients available and to test whether IVF outcomes are associated with inherited thrombophilias in a general IVF population.

Materials and methods

Literature search

Several databases including PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), Embase (<http://www.embase.com>), and Cochrane Library (<http://www.update-software.com/Cochrane/default.htm>) were retrieved up to 12 January 2016. The search terms were as follows: thrombophilia OR “factor V” OR “factor V Leiden” OR “activated protein C resistance” OR “factor II” OR “factor II mutation” OR “prothrombin” OR “prothrombin G20210A mutation” OR MTHFR OR “methylenetetrahydrofolate reductase” OR “protein C” OR “protein C deficiency” OR “protein S” OR “protein S deficiency” OR antithrombin OR “antithrombin deficiency”) AND (in vitro Fertilization) OR IVF OR “assisted reproduction technologies” OR ART. In addition, manual retrievals on related review and reference literature were also performed to include more researches in this meta-analysis.

Study selection

Studies were included if they met the following criteria: (i) cohort study; (ii) subjects were infertile women who undergo IVF; (iii) the case group was patients with inherited thrombophilia and the control group was patients without inherited thrombophilia; and (iv) results of the studies were clinical pregnancies rate and implantation rate.

Studies were excluded if one of the following existed: (i) case control study and cross-sectional study; (ii) review literatures, reports, comments, or letters; (iii) data which could not be used for statistical analysis.

Data extraction and quality evaluation

With the standard protocol, two investigators independently extracted the following data from the included studies: the first author's name; year of publication; country of origin; type of studies, age of subjects and cases; mutation factor of the genetic predisposition to the disease and the outcome of the study. Literature assess was performed according to the

Newcastle Ottawa scale (NOS) recommended by the Agency for Healthcare Research and Quality (AHRQ) [10]. Any disagreements were resolved by discussion between them or settled by a third reviewer.

Statistical analysis

Meta-analysis was carried out using Stata11.0 software. The relative risk (RR) and its 95 % confidence interval (CI) were calculated for effect index. Heterogeneity test was evaluated by Chi-square based on Q statistic [11] and I^2 statistics [12]. The random effects model was used to combine the data for the heterogeneous outcomes ($P < 0.05$ or $I^2 \geq 50$ %); otherwise, the fixed effects model was used [13]. A P value less than 0.05 was considered statistically significant.

Results

Characteristics of included studies

The process of study selection was shown in Fig. 1. Initially, a total of 1882 potentially relevant articles were retrieved from the databases (PubMed 481; Embase 1366; Cochrane Library 32). Then, 1503 articles were left after eliminating the duplicate publication, and then 1487 of them were excluded after screening the title and abstracts. As a consequence, 16 articles were left and 9 (3 not cohort study, 3 without related outcome, 2 with men subjects and 1 review) of them were excluded after screening the full text. No article was obtained with manual retrieval. Finally, 7 articles [14–20] were included in this meta-analysis (Table 1). These included studies were published between 2007 and 2015 with the ages of subjects between 30.9 and 36.2. Among them, 5 articles were prospective cohort study [14–16, 19, 20] and 2 articles were retrospective cohort [17, 18]. Mutations of the inherited thrombophilia included factor V Leiden (FVL), prothrombin gene G20210A mutation (PGM), 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T, MTHFR(A1298C) and activated protein C resistance (APCR). All of these 7 articles were high-quality studies (Table 2).

Merging quantitative data

The quantitative data were combined with the mutation factor, and differences of clinical pregnancy rate (CPR) and implantation rate (IR) in mutation group and control group were analyzed. As shown in Fig. 2, four studies [14, 17, 19, 20] reported MTHFR (C677T) mutation and the results showed that MTHFR (C677T) mutation in inherited thrombophilia was not associated with CPR. The homogeneity analysis ($P = 0.52$, and $I^2 = 0.0$ %) and effect size merge (RR = 1.05, 95 % CI 0.97–1.14, $P = 0.023$) showed that there was no significant difference in MTHFR (C677T) mutation between the

Fig. 1 Flow diagram of study selection

mutation group and control group. Three articles [14, 19, 20] reported MTHFR (A1298C) mutation and no heterogeneity ($I^2=0.0\%$, $P=0.39$) or significant difference ($RR=1.03$, 95 % CI 0.95–1.12, $P=0.50$) in MTHFR (A1298C) was observed between the two groups. Two articles [15, 20] reported FVL mutation and the result of heterogeneity test was $I^2=96.0\%$, $P<0.00001$, and no significant differences ($RR=1.57$, 95 % CI 0.47–5.27, $P=0.46$) were observed between the two groups. Two articles [18, 20] reported PGM mutation and the heterogeneity test results was $I^2=0.0\%$, $P=0.8$, and no significant difference ($RR=0.83$, 95 % CI 0.64–1.09, $P=0.18$) was observed. One study reported FVL/PGM mutation ($RR=1.14$, 95 % CI 0.62–2.12, $P=0.67$) and one reported FVL/APCR mutation ($RR=1.30$, 95 % CI 1.03–1.63, $P=0.03$).

In addition, IR in mutation group and control group were also analyzed, and the results showed that there was no significant difference in IR between the mutation group and control group (Fig. 3). As shown in Fig. 3, two articles [17, 20] reported MTHFR (C677T) mutation and no heterogeneity ($I^2=40.0\%$, $P=0.2$) or significant difference ($RR=1.05$, 95 % CI 0.83–1.33, $P=0.66$) was observed between the mutation group and control group. Meanwhile, 2 articles [15, 20] reported FVL mutation with good heterogeneity ($I^2=92.0\%$, $P=0.0006$) and no significant difference ($RR=1.57$, 95 % CI 0.55–4.47, $P=0.40$) were observed. In addition, one article [20] reported MTHFR (A1298C) ($RR=1.01$, 95 % CI 0.93–1.10, $P=0.77$), one [20] reported PGM ($RR=0.83$, 95 % CI 0.63–1.10, $P=0.20$) and one [16] reported FVL/PGM ($RR=1.31$, 95 % CI 0.82–2.09, $P=0.26$), and no significant differences of these mutation were found between the two groups.

Publication bias test

As the small number of included studies, the test efficiency of publication bias was low, and no publication bias existed in this study.

Discussion

Some systematic reviews on associations between IVF failure and inherited thrombophilias have been reported in previous studies. In this meta-analysis, seven articles published between 2007 and 2015 with the ages of subjects between 30.9 and 36.2 were included. The results showed that there were no significant differences in CPR or IR between the mutation group and control group.

A large number of articles suggest a higher prevalence of inherited thrombophilias in recurrent implantation failure compared with healthy parous women [7, 21, 22]. However, other studies have opposite outcomes. Gopel et al. [23] showed that compared with non-carriers, FVL mutation carriers are more prone to pregnancy in first IVF cycle. Compared with patients with FVL negative, the median number of unsuccessful transfers in pairs with FVL positive was lower. Our findings suggest that the improved IR acts as an important genetic advantage of the FVL mutation. In addition, women with FVL mutation have been found to have higher Hb levels, a lower incidence of life-threatening postpartum hemorrhage and lose less blood in menstruation [24].

The relationships between inherited thrombophilia and fertility are controversial. Coulam, et al. [5] reported that total gene mutation was associated with recurrent miscarriage. However, there

Table 1 The characteristics of the included studies in this meta-analysis

Author	Year	Country	Type of study	Factor	n (mutation/control)	Age (years)	Outcomes
Dobson	2007	USA	PC	MTHFR(C677T)	197 (101/96)	35.2/35.5	Clinical pregnancies
				MTHFR(A1298C)	196 (100/96)		
Rudick	2009	USA	PC	FVL	182 (3/179)	32.0/35.4	Implantation rate, clinical pregnancies
Marci	2012	Italy	RC	MTHFR(C677T)	273 (103/170)	33.8/34.6	Implantation rate, clinical pregnancies
Ricci	2011	Italy	PC	FVL/PGM	480 (30/450)	35.1/36.2	Implantation rate, clinical pregnancies
Steinvil	2012	Israel	RC	FVL/APCR	445 (35/410)	30.9 (4.1)*	Clinical pregnancies
				PGM	511 (16/495)		
D'Elia	2014	Brazil	PC	MTHFR(C677T)	82 (47/35)	32.0/31.0	Clinical pregnancies
				MTHFR(A1298C)	82 (38/44)	31.0/32.0	
Patounakis	2015	USA	PC	MTHFR(C677T)	1717 (919/798)	35.0 (4.4)*	Implantation rate, clinical pregnancies
				FVL	1717 (59/1658)		
				PGM	1717 (50/1667)		
				MTHFR(A1298C)	1717 (927/790)		

PC prospective cohort, RC retrospective cohort, FVL factor V Leiden, MTHFR 5,10-methylenetetrahydrofolate reductase, PGM prothrombin gene G20210A mutation, APCR activated protein C resistance

*mean age of all participants

was no association between recurrent miscarriage and frequency of homozygous mutations for factor V (G1691A; Leiden), factor V [H1299R (R2)], factor V (Y1702C), factor II prothrombin (G20210A), factor XIII (V34L), b-fibrinogen (455G>A), plasminogen activator inhibitor-1 (PAI-1) (4G/5G), human platelet antigen 1 (HPA1) (a/b9L33P), methylenetetrahydrofolate reductase (MTHFR) (C677T) or MTHFR (A1298C). A previous small case–control study has demonstrated that FVL could significantly increase the risk of infertility [25], but this association has not been observed in another study [26]. A role of thrombophilia has also been advised in recurrent IVF failure. In addition, a close relationship between thrombophilia caused by FVL and recurrent implantation failure has been found in a previous study [27]. In our study, no significant difference (RR = 1.57, 95 % CI 0.47–5.27, $P=0.40$) in the prevalence of FVL was found between mutation

group and control group, suggesting no associations between IVF and inherited thrombophilias.

In the current study, IR did not show a significant difference in mutation patient comparison with non-mutation carrier cases. A previous study has reported that thrombophilic mutations contribute to a greater chance of the embryo implanting [27]. Mutation carrier women may have a more favorable uterine for implantation. Consistent with our study, Ricci et al. [28] found that IR were not significantly different between carrier and non-mutation carrier women before and after adjustment for female age, infertility diagnosis and the number of IVF cycles. Thus, we conclude that inherited thrombophilias did not affect embryo implantation.

These meta-analysis results should be cautiously interpreted because there are still some limitations. (i) This meta-analysis included observational studies, and the associations between

Table 2 Methodological quality of cohort studies included in the meta-analysis

First author	Representativeness of the exposed cohort	Selection of the unexposed cohort	Ascertainment of exposure	Outcome of interest not present at start of study	Control for important factor or additional factor ^a	Outcome assessment	Follow-up long enough for outcomes to occur	Adequacy of follow-up of cohorts	Total quality scores
D' Elia	☆	☆	☆	☆	☆	☆	☆	☆	8
Dobson	☆	☆	☆	☆	☆	☆	☆	☆	8
Marci	☆	☆	☆	–	☆	☆	☆	☆	7
Patounakis	☆	☆	☆	☆	☆	☆	☆	☆	8
Ricci	☆	☆	☆	☆	☆	☆	☆	☆	8
Rudick	☆	☆	☆	☆	☆	☆	☆	☆	8
Steinvil	☆	☆	☆	–	☆	☆	☆	☆	7

A study could be awarded a maximum of one star for each item except for the item *control* for important factor or additional factor

^a A maximum of two stars could be awarded for this item

Fig. 2 Effect size of clinical pregnancies rate (mutation vs. control group) **Fig. 3** Effect size of implantation rate (mutation vs. control group)

genetic predisposition and IVF outcomes may be affected by confounding factors. In spite of the quality control and correction of the mixed factors including age and infertility, these factors can still bring bias to the results; (ii) Patients with inherited thrombophilias were included in the articles, and thrombophilias was not test in the current study; (iii) As embryos are not screened for aneuploidy, age is a confounding factor. However, age was not included in this meta-analysis; (iv) As the small number of studies, no published bias test was performed, and it is necessary to carry out more quality researches to verify this result.

In conclusion, our data support the concept that IVF outcomes are not associated with FVL, PGM, MTHFR (C677T), MTHFR (A1298C) and APCR mutation in inherited thrombophilias. However, there are still some problems to be not ignored, and it is necessary to design rigorous and large sample quality researches to verify the analysis results.

Acknowledgments Nantong science and technology project (HS2014012).

Youth Fund Project of Nantong Municipal Health Bureau (WQ2015068).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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