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Thrombophilias and adverse pregnancy outcome – A confounded problem!

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Summary

It was the objective of this study to analyse the influence of confounders, such as ethnicity, severity of illness and method of testing, in articles concerning the still moot relationship of thrombophilias to adverse pregnancy outcome (APO). Relevant case-control studies were identified using Medline and EMBASE databases between 1966 and 2006. Search terms were recurrent fetal loss, intrauterine fetal death, preeclampsia, HELLP-syndrome, eclampsia, fetal growth restriction, abruptio placentae, combined with maternal thrombophilias. Data was extracted from the articles per subgroup of APO regardless of confounder. These subgroups were tested if they fulfilled the heterogeneity testing criterion ($I^2 > 35\%$) to weigh the influence of the confounder. Confounders were selected and examined with Mantel-Haenszel method. Increased thrombophilia prevalence was confirmed in most adverse pregnancy outcomes. Ethnicity, genetic testing only and severity of illness were confounders in the various forms of APO. Stronger relationships between factor V Leiden and severity of disease were found in 2nd and 3rd trimester than 1st trimester recurrent fetal loss, in preeclampsia with: blood pressure ≥160/110 mmHg than ≥140/90 mmHg; proteinuria ≥5 grams per day than < 5 grams; onset before than after 28 weeks, in fetal growth restriction <3rd percentile than <5th, than <10th, and in earlier occurrence of abruptio placentae than 3rd trimester. In conclusion, reports on the prevalence of maternal thrombophilias and APO are influenced by various confounders, which are not always appropriately analysed. The differences we have identified reflect the differential impact of these confounders. These data emphasise the importance of more uniform research.

Keywords

Thrombophilia, pregnancy, confounders, illness severity

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Introduction

The influence of pregnancy on haemostasis and *vice versa* has been of great interest in obstetric medicine. A clear relationship between non-hereditary factors (antiphospholipid antibodies) and adverse pregnancy outcome has been previously confirmed, providing a paradigm for the hypothesis that an increased tendency to hypercoagulability leads to pregnancy complications (1–6). There is an ongoing proliferation of research attempting to clarify the association between hereditary thrombophilic disorders, including antithrombin deficiency, protein C deficiency, protein S deficiency, activated protein C resistance, factor V Leiden mutation, prothrombin gene G20210A mutation and the common polymorphisms (C677T and A1298C) of the gene for methylenetetrahydrofolate reductase (MTHFR), and adverse pregnancy outcome (APO).

Despite numerous publications, however, concerning the prevalence of such inherited thrombophilias in women with APO, the relationship remains moot, while the heterogeneity among study results is undeniable. In numerous reviews published with the aim of elucidating the relationship, only a few have mentioned the possible influence of confounders such as ethnicity (7–12). This review will focus on the heterogeneity between published studies with the aim of identifying and comparing confounders, so as to provide a clearer view on the relationships of thrombophilias to APO.

Methods

To study possible causes of heterogeneity in studies on thrombophilia and APO, articles were selected pertaining to the various thrombophilic disorders and using APO as an outcome measurement.

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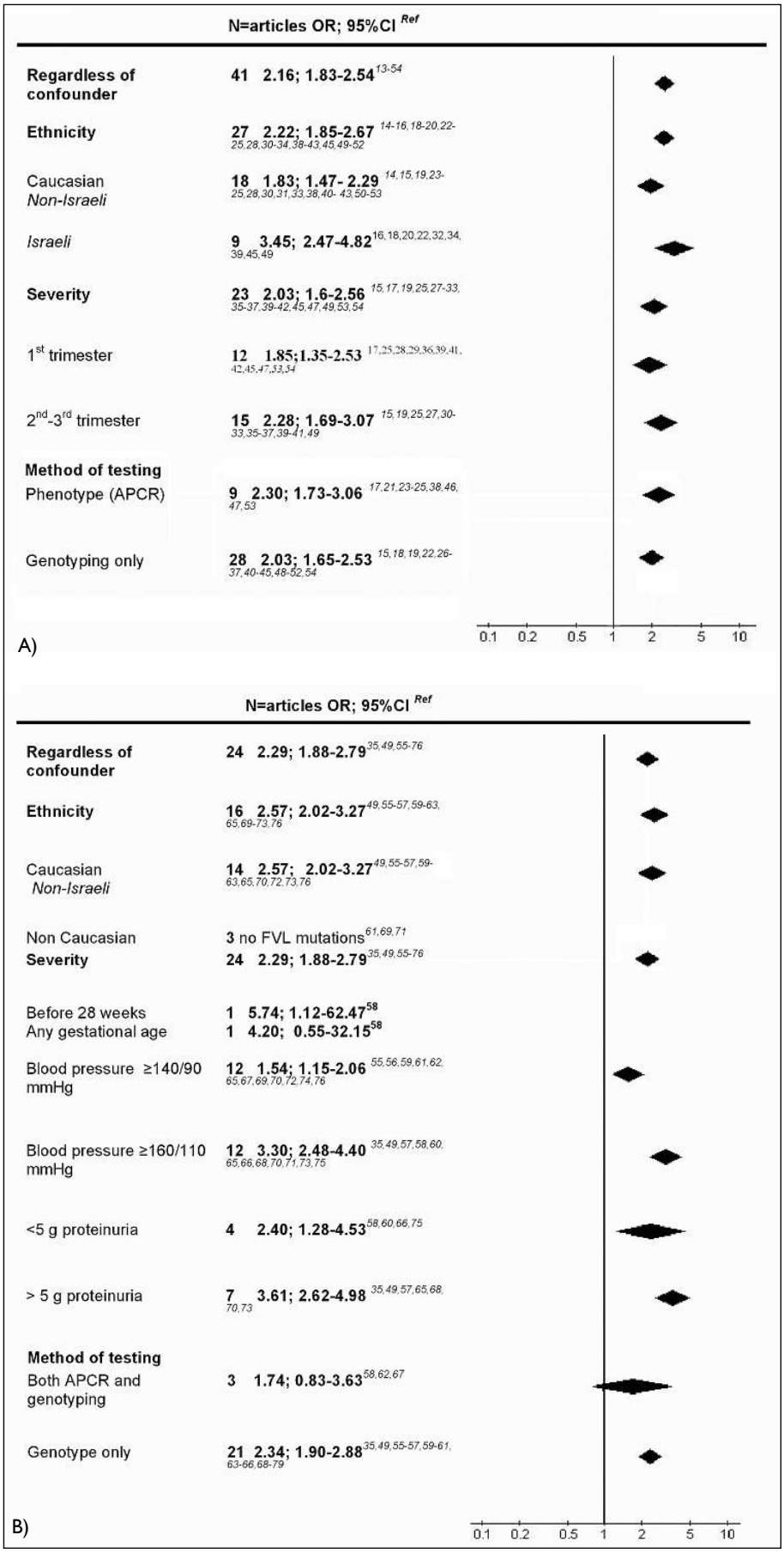

Figure 1: Relationships with FVL per adverse pregnancy outcome regardless of and regarding to confounders.
A) Recurrent fetal loss;
B) Preeclampsia;
C) Fetal growth restriction;
D) Abruptio placentae.

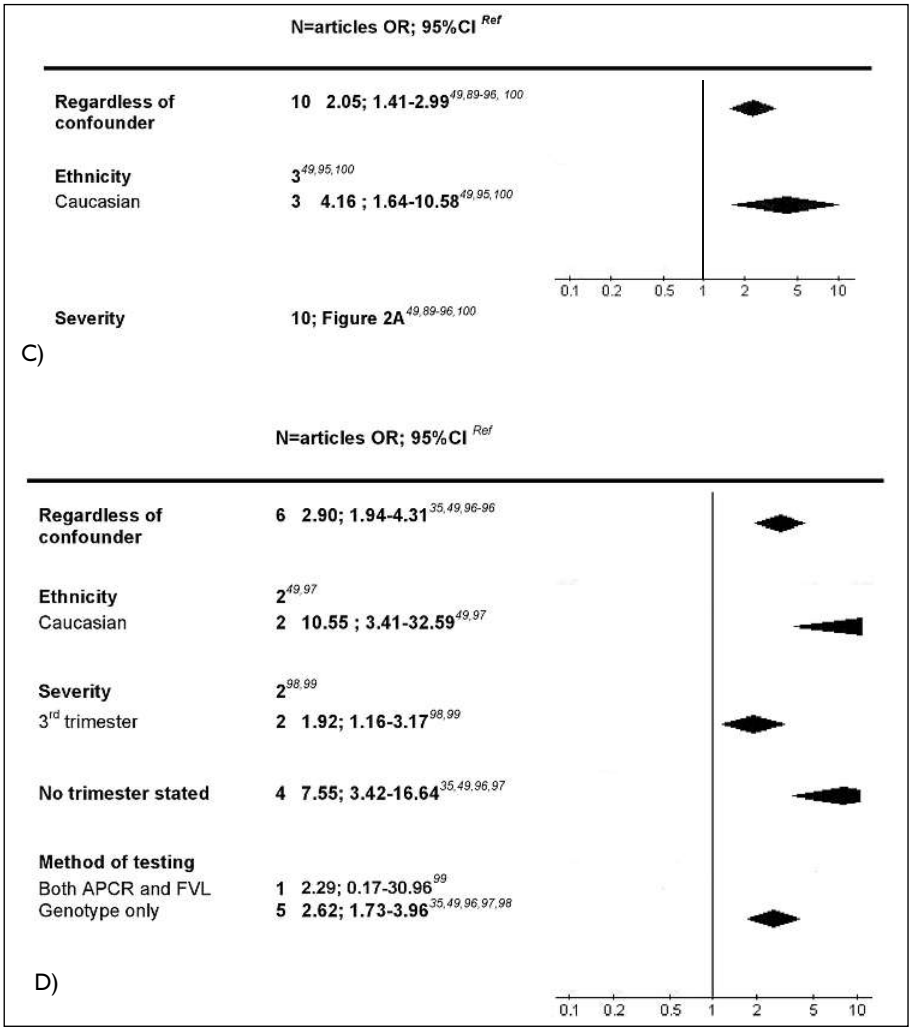

Figure 1: Continued.

Identification of relevant case-control studies

Case-control studies were identified by searching the Medline and EMBASE databases between 1966 and November 2006 for terms relating to recurrent fetal loss (RFL), intrauterine fetal death (IUD), preeclampsia (PE), HELLP-syndrome, eclampsia, fetal growth restriction (FGR), abruptio placentae (AP) and fetal thrombophilia, combined with antithrombin deficiency, protein C deficiency, protein S deficiency, activated protein C resistance (APCR), factor V Leiden mutation (FVL), prothrombin gene G20210A mutation (PGM), hyperhomocysteinemia (Hhcy), methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms, antiphospholipid antibodies, anticardiolipin antibodies (ACA) and lupus anticoagulant (LAC).

Only case-control studies published in the English language were accepted for meta-analysis. Their references were explored for other publications. Data was extracted by four reviewers (J. I. P. de Vries, J. J. Kalk, N. G. Janssen, W. J. Kist).

The set-up of the meta-analysis followed three steps. Firstly, all articles were categorized and analyzed per subgroup of APO (RFL, IUD, PE, FGR, AP) in relation to each thrombophilia regardless of confounder. Secondly, these subgroups were tested to see if they fulfilled the heterogeneity testing criterion ($I^2 > 35\%$) to weigh the influence of the various confounders (13). Finally, articles were selected which established a clear definition of the researched confounders so as to analyse the influence of the various confounders on the relationship between thrombophilias and APO, using RevMan software version 4.2.8 with the Mantel-Haenszel method for combining trials. The Mantel-Haenszel method is a statistical method for adjusting for confounding factors when analysing the relationship between a dichotomous outcome and a dichotomous risk factor.

Definition of potential confounders

1. Ethnicity: Articles were selected where the ethnic composition of their control and study groups was defined.
2. Severity of the illness: Articles were selected where the following categories were distinguished: recurrent fetal loss (RFL) during 1st trimester; RFL during 2nd and 3rd trimesters; preeclampsia (PE), defined by a blood pressure of ≥140/90 mmHg; severe PE, defined by a blood pressure of ≥160/110 mmHg, with proteinuria <5 grams per 24 hours; severe PE with proteinuria ≥5 grams per 24 hours; gestational age at delivery in preeclamptic women of <28 weeks; gestational age at delivery in preeclamptic women at any ges-

tation; fetal growth restriction (FGR) resulting in a birth weight below the 10th, 5th and 3rd percentiles respectively; FGR in combination with other APO; gestational age at birth in women with FGR; gestational age at birth in women with abruptio placentae (AP).

3. Method of testing: Articles were selected where one or more genetic polymorphisms associated with thrombophilia, e.g. FVL, (which can be ascertained at any time), had been tested for – "genetic testing", or where a functional test, e.g. APCR or Protein S deficiency, (which may be affected by pregnancy), had been performed – "functional testing", or where both genetic and functional tests had been performed.

Results of the meta-analyses

The search strategy revealed 98 case-control studies focused on the various subgroups of APO. Of all thrombophilic anomalies tested in these studies, only FVL, APCR, PGM, MTHFR and Hhcy met the criteria for testing potential confounders. The findings are presented below by subgroup of APO. Since FVL was tested in all subgroups, it is presented in several tables by subgroup of APO (Fig. 1A: recurrent fetal loss, B: preeclampsia, C: fetal growth restriction and D: abruptio placentae).

Recurrent fetal loss

Ethnicity

Forty-one articles were found analysing the relationship between RFL, intra-uterine death (IUD) and FVL (14–54). Out of these 41, 27 could be used in a meta-analysis of the ethnicity confounder. The 18 studies on non-Israeli Caucasian women (15, 16, 20, 24–26, 29, 31, 32, 34, 39, 41–44, 51–53) produced a weaker relationship (odds ratio [OR] 1.83: 95% confidence interval [CI] 1.47–2.29), and nine studies conducted in Israel (17, 19, 21, 23, 33, 35, 40, 46, 50) showed a stronger relationship (OR 3.45: 95% CI 2.47–4.82).

Severity of illness

Of the 41 articles mentioned, 23 could be used in a meta-analysis of severity of illness, which showed that, regardless of this particular confounder, there was a relationship with FVL (OR 2.03: 95% CI 1.61–2.56). The relationship was weaker in respect of 1st trimester loss (17, 25, 28, 29, 36, 39, 41, 42, 45, 47, 53, 54), when compared with loss in the 2nd and 3rd trimesters (15, 19, 25, 27, 30–33, 35–37, 39–41, 49).

Method of testing

Of the 49 articles on the relation between a thrombophilia and APO, 27 used genotyping and functional testing, 13 used genotyping only and seven used functional testing only.

In the nine studies that conducted an APCR assay (17, 21, 23–25, 38, 46, 47, 53), the relationship of APCR with APO was stronger than the relationship of FVL with APO in the 28 studies that performed a FVL assay alone (15, 18, 19, 22, 26–37, 40–45, 48–52, 54).

Preeclampsia

The subgroup of articles addressing PE and MTHFR C677T homozygous was the only subgroup that did not have an I^2 value of 35% or higher (I^2 18%), but was analyzed because of the large number of studies (19 out of 40).

Ethnicity

Twenty-four studies were identified studying the relationship between FVL and PE (35, 49, 55–76), of these 16 could be used in the meta-analysis addressing the issue of ethnicity. Fourteen articles that documented a Caucasian population (49, 55–57, 59–63, 65, 70, 72, 73, 76) showed a stronger relationship between FVL and PE than in the three studies on the association between FVL and PE in non-Caucasians (61, 69, 71) in which neither patients nor controls were shown to carry the mutation. Two of these studies used solely non-Caucasian populations (68, 70).

Nineteen articles were identified studying the relationship of PE and MTHFR C677T homozygous (35, 55, 59, 60, 64–66, 70, 77–87) and confirmed a significant relationship (OR 1.54: 95% CI 1.30–1.82). Of these 19 articles, 15 addressed the issue of ethnicity. Nine articles on Caucasian subjects (55, 59, 60, 65, 70, 80, 83, 84, 87) showed a stronger relationship of PE with MTHFR C677T homozygous (OR 1.68: 95% CI 1.37–2.07) than three articles on Asian subjects (78, 79, 86) where the relationship was not significant (OR 1.15: 95% CI 0.76–1.74). Three articles using African subjects (77, 81, 85) also did not demonstrate a significant relationship (OR 1.53: 95% CI 0.34–6.94).

Severity of illness

Of the 24 studies addressing the relationship between PE and FVL, half used a cut-off of ≥140/90 mmHg to define PE (55, 56, 59, 61, 62, 65, 67, 69, 70, 72, 74, 76), and the other half used a cut-off of ≥160/110 mmHg to define severe PE (35, 49, 57, 58, 60, 65, 66, 68, 70, 71, 73, 75). Those studies with the lower cut-off for blood pressure showed a weaker relationship between PE and FVL than the studies that used more severe hypertension to define the study population. Articles where proteinuria ≥ 5 grams per 24 hours was used as a criterion for severe PE (35, 49, 57, 65, 68, 70, 73) showed a stronger relationship of PE with FVL than those articles that used proteinuria <5 grams per 24 hours as the definition (58, 60, 66, 75).

Of the 19 articles addressing the relationship between PE and MTHFR C677T homozygous, the six studies that used ≥160/110 mmHg as a criterion for definition (35, 60, 65, 66, 70, 79) showed a stronger relationship (OR 1.77: 95% CI 1.32–2.38) than that in 13 articles that used a minimum blood pressure of ≥140/90 mmHg to define the diagnosis (55, 59, 65, 70, 77, 79, 81, 83–88) (OR 1.30: 95% CI 1.06–1.58). Two articles used both definitions (78, 79), dividing their cases into mild and severe PE groups.

One article (58) subcategorised severe PE by gestational age at delivery before versus at or after 28 weeks gestation. To ascertain the difference between making subgroups by gestational age at delivery and not doing so, we compared the group of women who delivered before 28 weeks with the total number of women. In women with APCR (OR 14.49: 95% CI 1.77–118.60), with FVL (OR 5.74: 95% CI 1.12–62.47), with Hhcy (OR 4.99: 95% CI 1.32–18.9) and with ACA ≥10 (OR 3.84: 95% CI 1.40–10.50), although not in women with ACA ≥20, where numbers were smaller, the ORs for severe PE were higher in the

early onset group compared to the total number of women in both study groups with APCR (OR 8.38: 95% CI 1.12–6.47), FVL (OR 4.20: 95% CI 0.55–32.15), Hhcy (OR 2.94: 95% CI 0.88–9.86) and with ACA ≥10 (OR 2.68: 95% 1.11–6.47 CI).

Method of testing

Of the 38 articles documenting the relationship between a thrombophilia and APO, seven used genotyping and functional testing, 25 articles used genotyping only and six articles used functional testing only.

Within the 24 articles addressing the relationship of PE with FVL, 21 studies that used genotyping alone (35, 49, 55–57, 59–61, 63–66, 68–76) showed a stronger association (OR 2.34: 95% CI 1.90–2.88) than the three studies in which both genotyping and APCR assay were used (OR 1.74: 95% CI 0.83–3.63) (58, 62, 67). One article shows the importance of APCR testing (55), since it reports a significant relationship between PE and APCR, whereas there was no association of PE with FVL.

Since all 17 articles addressing the relationship of PE with MTHFR C677T homozygous used genotyping alone, rather than using the phenotype of Hhcy, a confounder analysis could not be performed.

Fetal growth restriction

Ethnicity

Ten articles discussed the association of FGR with FVL regardless of confounder (49, 89–96, 100) and showed a significant relationship (OR 2.05: 95% CI 1.41–2.99). Three of these addressed ethnicity (49, 95, 100) and showed a much stronger association of FGR and FVL in the Caucasian group (OR 4.16: 95% CI 1.64–10.58). The same three articles also studied the association of FGR and PGM in respect of ethnicity, showing comparable re-

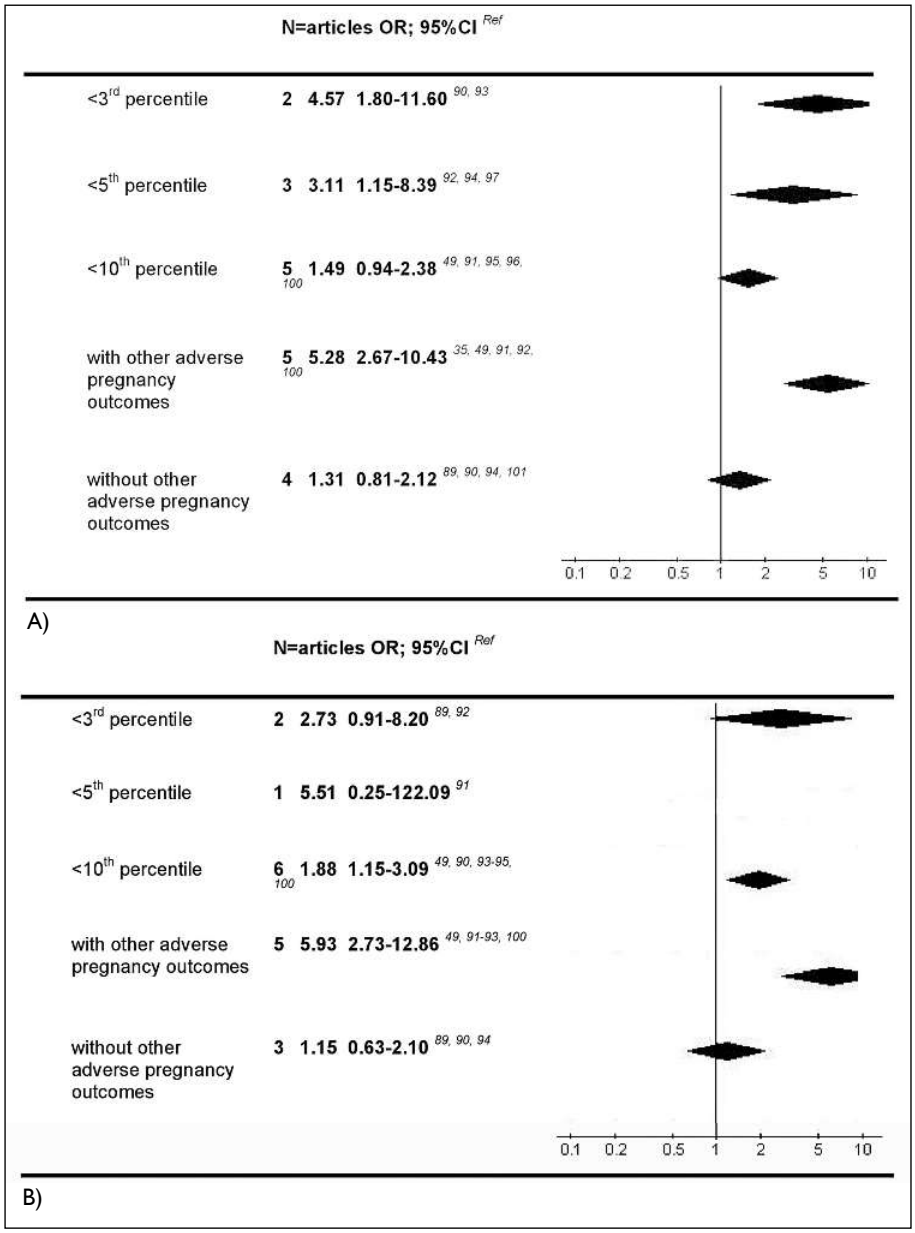

Figure 2: Birth weight percentiles and other adverse outcomes in women with a history of FGR in respect of FVL (A) and PGM (B).

sults for the Caucasian group (OR 3.79: 95% CI 1.78–40.40) compared with the data from all nine articles (49, 89–95, 100) analyzed regardless of confounder (OR 2.07: 95% CI 1.33–3.23).

Severity of illness

All ten articles discussing the relationship between FGR and FVL (49, 89–96, 100), and all nine articles considering FGR and PGM (49, 89–95, 100) were used in a meta-analysis to compare different incidences of the two genotypes in FGR below the 3rd, the 5th and the 10th percentiles. For FVL, there was a stronger relationship with the more profound degrees of FGR, whereas for PGM the only significant relationship was seen for FGR <10th percentile. A second meta-analysis was performed, comparing articles that used other adverse pregnancy outcomes as an exclusion criterion with those that did not, showed a stronger relationship of both FVL and PGM with FGR combined with other APO (see Fig. 2).

In relation to FGR and gestational age at delivery, one study identified a significant association between FVL and FGR in women who delivered at a gestational age of 22–26 weeks (92). In a second study (89), a significantly higher frequency of thrombophilias was noted in women with FGR who delivered at or after 37 weeks compared with women with FGR regardless of gestational age. This study did not, however, document which specific thrombophilias were linked to FGR at particular gestational ages.

Method of testing

Of the 12 articles on thrombophilia and APO five articles used genotyping and functional testing and seven used genotyping only.

In respect of the association between FGR and FVL, the only testing performed in all included articles was genotyping.

Abruptio placentae

Ethnicity

Six articles addressed the relationship of AP with FVL regardless of confounder (35, 49, 96–99) confirming a significant association (OR 2.90: 95% CI 1.94–3.31). Only two of these articles discussed ethnicity (49, 97), both articles dealing with Caucasian populations. The relationship was very strong in the Caucasian populations (OR 10.55: 95% CI 3.41–32.59), and much less so, although still significant, when no account of ethnicity was taken.

Severity of illness

With regard to the confounder of gestational age at delivery, only two out of six articles (98, 99) used 3rd trimester as a part of their definition of AP in relation to FVL, giving a much less significant relationship (OR 1.92: 95% CI 1.16–3.17) compared with the other four articles (35, 49, 96, 97) that did not account for gestational age at delivery (OR 7.55: 95% CI 3.42–16.64).

Method of testing

Of the 12 articles on thrombophilia and abruptio two used phenotype only, four used genotype only and six used both. As for the relationship between AP and FVL, five articles used genotyping only revealing OR 2.62: 95% CI 1.73–3.96, and only one article used both phenotyping and genotyping.

Discussion

This is the first study focusing on the effect of confounders in the analysis of thrombophilia examined after APO. The meta-analyses reveal the confounding influence of ethnicity, severity of disease and genotyping alone versus genotyping and phenotyping combined in all the examined varieties of APO.

When considering the prevalence of genetic thrombophilia the knowledge of ethnicity will allow adjustment of results for different populations: for example, the high prevalence of FVL in Caucasians, and especially Israelis, needs to be considered in relation to the incidence of recurrent fetal loss. The confounding influence of ethnicity was not, however, limited to FVL alone but could also be demonstrated in respect of MTHFR and PGM, both examined in relation to preeclampsia.

Also striking was the varied influence of the severity of the disease in those forms of APO we could examine. Firstly, gestational age at birth revealed a higher incidence of FVL in women with recurrent fetal loss in the 2nd and 3rd trimester than in those with recurrent 1st trimester loss. This is not surprising considering the absence of a functional intervillous space up to 9–10 week's gestationa, i.e. it is highly unlikely that a thrombotic event could cause embryonic loss prior to nine week's gestation. In preeclampsia and abruptio placentae the confounding influence seems to be the opposite, the earlier the gestational age at birth the higher the incidence of FVL. In FGR both were found: one study described more FVL at birth less than 26 weeks than at other gestational ages, while another study found more FVL at delivery beyond 37 weeks compared with delivery at other gestational ages. An explanation could be that, although the aetiology of FGR is varied, a large part is due to utero-placental insufficiency with the same origin (i.e. thrombotic vasculopathy) as for preeclampsia.

Secondly, the severity in growth restriction showed the same influence in both women with pregnancies complicated by FGR or preeclampsia; the more severely growth restricted fetuses or the earlier the onset of PE, the higher the incidence of FVL.

Thirdly, the elevation of the blood pressure had confounding influence; the higher the blood pressure, the higher the incidence of FVL.

Fourthly, the degree of proteinuria had similar confounding influence; the more severe the proteinuria, the higher the incidence of FVL

Fifthly, the studies on FGR that did not exclude other adverse pregnancy outcomes had a higher incidence of thrombophilic factors FVL and PGM than those that did. This leads to the suggestion that multiple adverse pregnancy outcomes may be related to a higher incidence of FVL.

Genetic testing without functional testing limits the information on the thrombophilic status of the subjects. This was found for FVL and APCR in all forms of adverse pregnancy outcome. From the work of Lachmeyer et al. (101) we know the limited correlation between MTHFR and hyperhomocysteinemia. This strengthens the need to elucidate the full thrombophilia status of the patient, including a broad spectrum of functional clotting tests (e.g. proteins S and C, antithrombin and APCR). Since

some of these factors may be influenced by pregnancy (including the postpartum period), the analyses need to be performed at a suitable time thereafter.

A limitation of this study is the lack of uniformity among articles, resulting in high heterogeneity. This was especially the case in those articles relating to abruptio placentae, diminishing the power of the confounder meta-analysis, since relatively few articles could be used in each confounder analysis.

When confounders were analyzed, significant differences became evident. To assess the true influence of a single confounder, every other variable in the comparison should ideally be the same. In practice this rarely happens. The differences in ORs between the overall meta-analyses and the relevant confounder-based meta-analyses might therefore not be solely due to the influence of the investigated confounder. They do, however, give an indication of the degree of impact of the various confounders and provide an incentive for more uniform and well-defined research. For example, heparin is increasingly used for prophylaxis of pregnancy-associated morbidity in pregnant women with thrombophilia (102). Two randomised controlled trials on the effect of heparin in women with unexplained first and second trimester recurrent miscarriages did find a beneficial effect (103, 104). The trial authors, however, did not discuss whether or not the effect was related to thrombophilia, nor did they consider if the consequence is that even recurrent first trimester miscarriages have to be treated. The uncertainty about the role of thrombophilia is highlighted in that, although one trial (103) studied women with antiphospholipid antibodies (without knowledge of other thrombophilic factors), the other trial (104) found the effect to occur in women even in absence of antiphospholipid antibodies or other thrombophilia factors. The uncertainty about the consequence of treatment of recurrent first trimester miscarriages is increased, inasmuch as both trials lumped together their data from first and second trimester miscarriages.

Conclusion

The prevalence of maternal thrombophilias and adverse pregnancy outcome has been demonstrated to be influenced by factors of ethnicity, severity of disease and methods of testing. Important influencing factors on the severity of disease are the gestational age at birth, growth restriction, hypertension, proteinuria and multiple adverse pregnancy outcomes. This study has tried to address the knowledge of these aspects and thus to enhance the prospect of uniform research in the future. We strongly suggest that the growing number of randomised controlled trials on beneficial effect of low-molecular-weight heparin will careful describe at least the three mentioned confounders: ethnicity, severity of disease and the genotype and phenotype of the thrombophilia parameters, in relation to any adverse outcome of pregnancy to prevent premature introduction of treatment.

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