Recurrent miscarriage and the subsequent risk of cardiovascular disease

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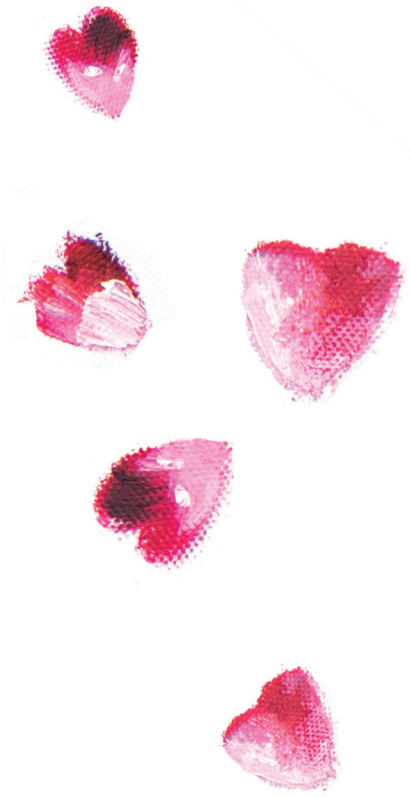

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Genetic polymorphisms in recurrent miscarriage: a meta-analysis

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Abstract

Background The underlying cause of recurrent miscarriage remains often unexplained. A multifactorial etiology, including uterine anomalies, endocrine and metabolic factors, maternal autoimmune disorders, thrombophilia, toxic factors and genetics is assumed. Currently it is still unclear which specific genes are involved. The aim of this study was to investigate systematically which genetic variants are reproducibly associated with recurrent miscarriage.

Methods A systematic review and meta-analysis was performed. PubMed, Embase and Web of Science were searched (till 7 October 2014) for case-control studies that assessed the association between genetic variants and recurrent miscarriage (defined as two or more unexplained miscarriages). The control groups consisted of women with at least one successful pregnancy and no miscarriages. Genetic variants significantly associated with recurrent miscarriages in at least two independent studies were considered as reproduced variants and were included. The association between genes and recurrent miscarriages was assessed at the allele level and a pooled odds ratio was estimated in a random-effects model. A subgroup analysis was performed for the association between genetic variants and three or more consecutive miscarriages. I^2 statistics was reported. Egger test was used to check funnel plot asymmetry.

Results The literature search yielded 4050 articles; a total of 241 studies were included. We identified 25 reproduced genetic variants, of which 16 remained significantly associated with recurrent miscarriage in a random-effects meta-analysis. These variants were in the following genes: F2, FV, FXIIIa, HLA-G, IL10, IL18, MTHFR (two variants), NOS3, PAI1 , STAT3, TNFA (two variants) and VEGFA (three variants). Odds ratios for these 16 variants ranged from 1.21 to 2.64. Eight variants were significantly associated with three or more consecutive miscarriages, in the following genes: FV, IFNG, MTHFR, NOS3, TNFA (two variants) and VEGFA (two variants).

Conclusions This meta-analysis found 16 genetic variants associated with recurrent miscarriage. Major involved pathways seem to be: coagulation and fibrinolysis, immunology and inflammation and angiogenesis. Unravelling mechanisms by which these genetic variants affect the risk of recurrent miscarriage can reveal potential targets in the search for treatment. This meta-analysis also suggests that recurrent miscarriage and cardiovascular disease share genetic risk factors.

Keywords: genetic variants, recurrent miscarriage, risk-factors, cardiovascular disease

Introduction

Spontaneous miscarriage is the most common complication of pregnancy. About 15% of clinically recognized pregnancies end in a miscarriage (Rai and Regan, 2006;Wilcox, Weinberg *et al.*, 1988). The definition of recurrent miscarriage varies between two and three and consecutive versus non-consecutive miscarriages (Kolte, Bernardi *et al.*, 2015). Generally, the definition of three or more consecutive miscarriages prior to the 22nd week of gestation is used (Stirrat, 1990) which affects 0.5-3% of all fertile couples (Jivraj, Anstie *et al.*, 2001). In more than 50% of the cases, the underlying cause remains unexplained after diagnostic tests (Porter and Scott, 2005). Etiologic factors which are assumed to be involved in the pathogenesis of recurrent miscarriage include genetic disorders, uterine anomalies, endocrine factors, maternal autoimmune disorders, metabolic disorders, maternal thrombophilia, obesity and toxic factors, such as smoking and alcohol consumption (Branch, Gibson *et al.*, 2010). Research suggests a multifactorial etiology with a role for genetics (Christiansen, Steffensen *et al.*, 2008;Kolte, Nielsen *et al.*, 2011). An increase in the risk of spontaneous miscarriage was seen in family of women with recurrent miscarriage compared to normal control subjects (Christiansen, Mathiesen *et al.*, 1990;Kolte, Nielsen *et al.*, 2011;Miskovic, Culic *et al.*, 2012;Nybo Andersen, Wohlfahrt *et al.*, 2000). In addition, a case-control study showed that women with two or more unexplained recurrent miscarriages more often had a family history of recurrent miscarriage compared to healthy control subjects, RR 3.2 (95%CI 1.3-8.1) (Parazzini, Bocciolone *et al.*, 1991). However, it is still unclear what specific genes are involved and what individual variants contribute to the development of recurrent miscarriages. Polymorphisms have been investigated in almost 90 different genes (Rull, Nagirnaja *et al.*, 2012).

The aim of this study was to give a systematic overview of the genetic variants associated with recurrent miscarriage. A meta-analysis was performed to assess the pooled effect of the genetic variants that are repeatedly investigated and that are significantly associated with recurrent miscarriage in at least two of the performed studies.

Methods

Literature search

The databases PubMed, Embase and Web of Science were searched in collaboration with a trained librarian (last updated 7 October 2014). Terms used in the search strategy were ‘Recurrent Miscarriage, ‘Habitual Abortion’, ‘Pregnancy Loss’, ‘Polymorphisms, or ‘Genes’. All relevant keyword variants were used, and database specific adaptation of the key terms was performed. In addition, names of specific genes and polymorphisms which are known to be related to preeclampsia (Buurma, Turner *et al.*, 2013) were added

to the search strategy. The only limitation used in the search was language; studies had to be published in English or Dutch. References of related systematic reviews were checked for additional articles.

Eligibility criteria

We searched for case-control studies comparing genetic variants between women with recurrent miscarriage and controls. Inclusion criteria were: 1. Recurrent miscarriage defined as two or more unexplained miscarriages (irrespective whether consecutive or not) in first and/or second trimester; 2. The control group consisted of women who had at least one successful pregnancy and no miscarriages. If a subset of subjects in a study suited the inclusion criteria, only the subset was included. In case of overlapping study populations only the study with the most extensive data was included, to avoid duplication.
All titles and abstracts were reviewed by two researchers (M.W. and A.V. and a subset by M.W. and A.B.), who independently assessed if the study compared at least one genetic variant between women with and without recurrent miscarriage. Genetic variant studies were screened for whether they met the inclusion criteria and whether the investigated genetic variant showed a significant association ($P<0.05$ was defined as significant) at the allelic and/or genotypic level with recurrent miscarriage. When a genetic variant was found to be significantly associated with recurrent miscarriage in at least two studies, that variant was considered a reproduced genetic variant. Finally, only studies that investigated any of these reproduced genetic variants were included in the meta-analysis.

Data extraction

Studies that met the inclusion criteria were entered into separate databases by two researchers (M.W. and A.V.) independently. These two databases were compared, differences were discussed until consensus was reached and allele frequencies were extracted. In case of missing data, at least two attempts were made to contact the corresponding author. When neither the published report nor the corresponding author provided sufficient data to calculate an odds ratio at the allele level or give assurance that the study met the inclusion criteria, the study was excluded.

Statistical analyses

Pooled odds ratio's (OR) with 95% confidence intervals (CI) were calculated at the allele level for the association between reproduced genetic variants and recurrent miscarriage. Data were pooled using a random-effects-model to take into account between-study heterogeneity. I^2 was reported, which reflects the percentage of total variation across studies due to heterogeneity rather than due to change (Higgins, Thompson *et al.*, 2003). Funnel plots were generated for all reproduced genetic variants. Egger test (Egger, Davey *et al.*, 1997) was used to check funnel plot asymmetry (test for small study effect) which

may reflect publication bias. This test was only used when 10 or more studies were included to investigate a genetic variant, because otherwise test power is too low to distinguish chance from real asymmetry (Sterne, Sutton *et al.*, 2011). If funnel plot asymmetry existed and between study heterogeneity was evident a comparison between a random-effects-model and a fixed-effects-model was made. A subgroup analysis was performed for studies which included women with three or more consecutive miscarriages, applying the previously described analyses. All analyses were performed using STATA (StataCorp. 2011. Stata Statistical Software, Release 10, College Station, TX, USA: StataC).

Results

The initial literature search yielded 4050 articles. Finally 129 articles, comprising 241 studies, were included in this meta-analysis (Figure 1). Altogether, 25 reproduced genetic variants in 18 genes were described. Included articles were published between 1997 and 2014. The number of studies per genetic variant ranged from 2 to 38, the number of cases per study ranged from 5 to 1000. A significant association for 16 of the 25 reproduced genetic variants and recurrent miscarriage was found in a random-effects meta-analysis. These variants were in the following genes: F2, FV, FXIIIa, HLA-G, IL10, IL18, MTHFR (two variants), NOS3, PAI1 , STAT3, TNFA (two variants) and VEGFA (three variants) (Figure 2A-B). Odds ratio's ranged from 1.21 to 2.64, no significant protective effects were found (Table 1).
A subgroup analysis was performed for studies which included women with three or more consecutive miscarriages; 19 reproduced genetic variants could be included. A significant association between 8 variants and three or more consecutive miscarriages was found in the random-effects meta-analysis in the following genes: FV, IFNG, MTHFR, NOS3, TNFA (two variants) and VEGFA (two variants) (Table 2) (Figure 2C-D). The references of the included studies per variant are described in Table 3. Characteristics and references of all included studies, forest and funnel plots for all individual genetic variants are provided in the supplementary data.

Genetic variants involved in the renin–angiotensin system
Eleven studies investigating the *angiotensin converting enzyme (ACE) insertion/deletion (I/D)* polymorphism *(rs1799752)* were included in this analysis, resulting in a pooled OR of 1.20 (95% CI 0.95-1.53). In the subgroup analysis of women with three or more consecutive miscarriages, 4 studies were included, resulting in a pooled OR of 0.85 (95% CI 0.72-1.02).

Genetic variants involved in lipid metabolism
Five studies were included in the *apolipoprotein E (APOE) (rs429358)* analysis. E3 versus E4 allele (minor allele) was included in the meta-analysis resulting in a pooled OR of 3.09(95% CI 0.93-10.24).

Genetic variants involved in coagulation and fibrinolysis

Seven reproduced genetic variants in five genes concerning coagulation and fibrinolysis were included in the meta-analysis, six variants remained significantly associated with recurrent miscarriage following meta-analysis. The variant *factor 2 (F2) G20210A (rs1799963)*, also known as prothrombin mutation, was investigated in 26 studies resulting in an OR of 1.94 (95%CI 1.23-3.06). A subgroup analysis was performed for studies defining cases as three or more consecutive miscarriages for *F2 G20210A* resulting in an OR of 1.62 (95%CI 0.82-3.19), including 14 studies. *Factor V Leiden (FVL) (rs6025)* was the most frequently investigated polymorphism regarding recurrent miscarriages. A total of 38 studies were included, resulting in an OR of 1.88 (95%CI 1.47-2.40). For the subgroup three or more consecutive miscarriages 21 studies were included with a pooled OR of 1.79 (95%CI 1.32-2.42) The variant factor XIIIa (*FXIIIa) Val34Leu (rs5985)* was investigated in 8 studies and was associated with recurrent miscarriage; pooled OR of 1.58 (95%CI 1.04-2.41). Three studies were included in the subgroup analysis for three or more consecutive miscarriages, resulting in an OR of 1.36 (95%CI 0.43- 4.28).

Two variants in the methylenetetrahydrofolate reductase (MTHFR) gene were included. The *MTHFR A1298C* polymorphism (rs1801131) was investigated in 14 studies resulting in an OR 1.34 (95%CI 1.05-1.72). The subgroup analysis for three or more consecutive miscarriages resulted in an OR of 1.13 (95%CI 0.91-1.40), including 7 studies. The *MTHFR C677T* variant *(rs1801133)* was investigated in 32 studies and was associated with recurrent miscarriage with an OR of 1.28 (95%CI 1.12-1.47). The subgroup analysis for three or more consecutive miscarriages showed a comparable result. The variant *plasminogen activator inhibitor-1 (PAI1) -675 4G/5G (rs1799889)* was investigated in 14 studies and was associated with recurrent miscarriage with a pooled OR of 1.29 (95%CI 1.05-1.57). The variant *protein Z (PZ) intron F G79A (rs3024718)* was not associated with recurrent miscarriage after meta-analysis, including 4 studies, with a pooled OR of 0.88 (95%CI 0.38-2.04).

Genetic variants involved in inflammation and immunology

Eleven reproduced variants in seven genes involved in inflammation and immunology were included, five remained significantly associated with recurrent miscarriage after meta-analysis. The only reproduced genetic variant concerning human leukocyte antigen (HLA) comprised HLA-G. Eleven studies investigated the *HLA-G 14bp I/D* variant *(rs1704)*. A pooled OR of 1.21 (95%CI 1.01-1.45) was found in the meta-analysis. *Interferon-gamma (IFNG) 874A/T (rs2430561)*, reported in 5 studies, was not associated with recurrent miscarriage after meta-analysis with an OR 1.12 (95%CI 0.82-1.54). However, in the subgroup analysis for three or more consecutive miscarriages an association was found; pooled OR 1.39 (95% CI 1.14- 1.70). The variant *Interleukin (IL)1B -511C/T (rs16944)* was investigated in 4 studies resulting in an OR of 1.17 (95%CI 0.79- 1.73). Four studies investigated the variant *IL6 -174G/C (rs1800795)* resulting in an OR of 1.74 (95%CI 0.96-3.15).

Three variants concerning IL10 were included. The variant *IL10 -592C/A (rs1800872)* was investigated in 7 studies, a pooled OR of 0.84 (95%CI 0.63-1.12) was found. Similar results were found in the subgroup analysis regarding three or more consecutive miscarriages, including 4 studies. *IL10 -819C/T (rs1800871)* was investigated in 4 studies resulting in a pooled OR of 1.22 (95%CI 0.93-1.60). Similar results were found in the subgroup analysis regarding three or more consecutive miscarriages, including 3 studies. *IL10 -1082A/G (rs1800896)* was investigated in 6 studies and was associated with recurrent miscarriage with an OR of 1.25 (95%CI 1.02-1.54). In the subgroup analysis, three or more consecutive miscarriages; a pooled OR of 1.23 (95% CI 0.86-1.76) was found including 3 studies. The variant *IL18 -656C/A (rs1946519)* was investigated in only 2 studies and was associated with recurrent miscarriage with an OR of 1.92 (95%CI 1.61-2.30). Three variants in the tumour necrosis factor –alpha(TNFA) gene were included. Five studies concerned the *-238G/A variant (rs361525)*; a pooled OR of 1.37 (95%CI 0.88-2.14) was found. However, a significant association was found for the subgroup with three consecutive miscarriages (including 3 studies); pooled OR 1.52 (95%CI 1.21-1.92). *TNFA -308G/A (rs1800629)* was investigated in 7 studies, a pooled OR of 1.46 (95% CI 1.17-1.84) was found. A similar result was found in the subgroup analysis for three consecutive miscarriages, including 4 studies. *The TNFA -1031C/T (rs1799964)* variant was investigated in 2 studies and associated with recurrent miscarriage, pooled OR 2.64 (95%CI 1.90-3.66).

Genetic variants involved in oxidative stress

The variant *NOS3 Glu298Asp (rs1799983)* was investigated in 8 studies resulting in a pooled OR of 1.53 (95% CI 1.16-2.01). Seven of these studies included women with three consecutive miscarriage, pooled OR 1.65 (95%CI 1.25-2.17)

Genetic variants involved in oncogenesis

The variant *signal transducer and activator of transcription 3 (STAT3) rs1053004* was investigated in only 2 studies and remained associated with recurrent miscarriage after meta-analysis with a pooled OR of 1.51 (95%CI 1.30-1.75).

Genetic variants involved in angiogenesis

Three reproduced variants in the vascular endothelial growth factor A(VEGFA) were included and all remained associated after meta-analysis. *VEGFA -583T/C (rs3025020)* was investigated in 3 studies and was associated with recurrent miscarriage with a pooled OR of 1.75 (95%CI 1.50-2.03). All the available studies included women with three or more consecutive miscarriages. *VEGFA 936C/T (rs3025039)* was reported in 9 studies resulting in an OR of 1.35 (95%CI 1.09-1.68). A similar result was found in the subgroup analysis for three or more consecutive miscarriages, including 5 studies. The *VEGFA -1154G/A (rs1570360)* variant was reported in 10 studies resulting in an OR of 1.24 (95%CI 1.02-1.50).

Discussion

In this meta-analysis 16 genetic variants were associated with recurrent miscarriage. Meta-analysis of several individual genetic variants has been performed previously in relation to recurrent miscarriage. However, this is the first meta-analysis which gives a complete overview for all genetic variants which were reproducibly significantly associated with recurrent miscarriage. These more robust data could lead to improved understanding of the pathogenesis of recurrent miscarriage and could help to find the right direction for future treatment options.

Strengths/Limitations

This meta-analysis included only reproduced genetic variants, defined as variants that were at least twice independently significantly associated with recurrent miscarriage. This method was described before (Buurma, Turner *et al.*, 2013;Mooyaart, Valk *et al.*, 2011) and aims to minimize the number of false-positive results. However, it is possible that variants which were only described in studies with small sample size and lack of power were missed using this method. Publication bias is a concern in all meta-analyses. Overestimation of effect is possible due to the fact that studies with a non-significant effect are less likely to be published. For this reason Egger tests were performed, which showed no evidence of funnel plot asymmetry. Of note, we only performed this test for analyses including 10 or more studies as recommended by Sterne et al (Sterne, Sutton *et al.*, 2011).

Criteria for study inclusion and exclusion are critical parts of a meta-analysis and can affect results. Recurrent miscarriage is a very heterogenic condition and as described in the introduction the definition of recurrent miscarriage varies in the literature between two and three and consecutive versus non-consecutive miscarriages (Kolte, Bernardi *et al.*, 2015). To reduce the clinical heterogeneity we performed a subgroup analysis for studies which included women with three or more consecutive miscarriages. To further reduce this heterogeneity we included only studies that comprised women with pregnancy loss before the third trimester and the miscarriages had to be unexplained. However, bias is still possible due to the variations in examinations performed to make the diagnosis 'unexplained recurrent miscarriage'. All control women had to be parous women (with no miscarriage), to be sure that all women had been at risk to miscarry. Despite our efforts, statistical heterogeneity was evident for several investigated variants (Table 1 and 2).

Comparisons with literature

Genetic variants in the following genes were associated with recurrent miscarriage in this meta-analysis: F2, FV, FXIIIa, HLA-G, IL10, IL18, MTHFR (two variants), NOS3, PAI1 , STAT3, TNFA (two variants) and VEGFA (three variants). Three main groups or pathways can be distinguished; variants concerning coagulation and fibrinolysis (*F2 G20210A,*

FVL, FXIIIa Val34Leu, MTHFR A1298C, MTHFR C677T, PAI1 -675 4G/5G), variants concerning immunology and inflammation (*HLA-G 14bp I/D, IL10 -1082A/G, IL18 -656C/A, TNFA -308G/A, TNFA -1031C/T*) and variants involved in angiogenesis (*VEGFA -583T/C, VEGFA 936C/T, VEGFA -1154G/A*).

Six genetic variants involved in coagulation and fibrinolysis remained associated with recurrent miscarriage. Inherited thrombophilia is an important topic of research on women with recurrent miscarriage. The major heritable forms of thrombophilia are *F2 G20210A* and *FVL* polymorphisms. Women with inherited thrombophilia have been shown to be at increased risk not only of thromboembolism, but also of complications of pregnancy including preeclampsia and fetal loss (Walker, 2000). *MTHFR A1298C* and *MTHFR C677T* polymorphism remained associated with recurrent miscarriage after meta-analysis. Both polymorphisms reduce the activity of the MTHFR enzyme, causing elevated concentration of homocysteine. Increased homocysteine concentration is associated with neural tube defects and has led to the hypothesis that high concentrations of homocysteine might be embryotoxic (Zetterberg, 2004). Hyperhomocysteinemia is related to recurrent miscarriage (Steegers-Theunissen, Boers *et al.*, 1992;Wouters, Boers *et al.*, 1993). PAI1 regulates fibrinolysis, the 4G deletion allele is associated with high PAI1 plasma levels (Burzotta, Di *et al.*, 1998). An association between the *PAI1 -675 4G* deletion and recurrent miscarriage was found in our meta-analysis with an OR of 1.29, and a 95%CI of 1.05-1.57. The meta-analysis published in 2013 by Su et al (Su, Lin *et al.*, 2013) did not find a significant association between PAI1 and recurrent miscarriage: OR 1.44 (95%CI 0.97-2.14). This is probably due to the number of included studies: we were able to include 14 studies, compared to 11 studies in the meta-analysis performed by Su et al.

It is assumed that HLA-G plays an important role in the maternal-fetal interface (Kovats, Main *et al.*, 1990). HLA-G modulates cytokine secretion to induce immunotolerance, regulates trophoblast invasion for implantation and contributes to vascular remodelling of spiral arteries for pregnancy maintenance (Roussev and Coulam, 2007). Lower circulating levels of soluble(s) HLA-G during the first trimester were found in women with recurrent pregnancy loss versus healthy pregnant controls (Jassem, Shani *et al.*, 2012). We found a pooled OR of 1.21 (95%CI 1.01-1.45) between *HLA-G 14bp* and recurrent miscarriage, although significant heterogeneity was evident across studies (I^2=57.0%). No significant association was found in our subgroup analysis for three or more consecutive miscarriages, likely because of the small number of included studies (4 studies). This result is comparable with the very recently published meta-analysis by Meuleman et al (Meuleman, Lashley *et al.*, 2015).

A significant association between *IFNG 874A/T* and recurrent miscarriage was only found in our meta-analysis including three or more consecutive miscarriages. IFNG is a pro-inflammatory cytokine, produced by type 1 T-helper cells which activate macrophages and are responsible for cell-mediated immunity and phagocyte-dependent protective

responses (Romagnani, 1999). Significantly higher levels of IFNG were found in women with recurrent miscarriage compared to women with a normal pregnancy (Raghupathy, Makhseed *et al.*, 1999;Wilson, Jenkins *et al.*, 2004). A meta-analysis performed in 2008 (Bombell and McGuire, 2008) showed a comparable result (OR 1.29), although they did not find a significant association. Our association likely turned out to be significant because we were able to include the large study performed by Parveen et al (Parveen, Shukla *et al.*, 2012) (which found an association: OR 1.49 (1.13-1.95)).

One of the three included polymorphisms in the IL10 gene remained significantly associated with recurrent miscarriage; *IL10 -1082A/G* (OR 1.25 (95%CI 1.02-1.54)). IL10 is known to selectively suppress Th1-mediated cellular responses by inhibiting the production of inflammatory cytokines (IFNG and TNFA). It has been proposed that a decrease in production of IL10 is associated with recurrent miscarriage and increased production is associated with normal pregnancy (Hill, Polgar *et al.*, 1995;Kamali-Sarvestani, Zolghadri *et al.*, 2005;Makhseed, Raghupathy *et al.*, 2000). However this is still controversial; a study found higher IL10 levels at miscarriage than during normal pregnancy (Vassiliadis, Ranella *et al.*, 1998). Our results suggest that women with recurrent miscarriage may have a genetic predisposition to secrete higher levels of IL10. Further research is needed to clarify the role of IL10 in recurrent miscarriages.

A closely related pro-inflammatory cytokine is IL18, also known as IFNG inducing factor (Al-Khateeb, Sater *et al.*, 2011;Boraschi and Dinarello, 2006). We found a significant association between *IL18 -656C/A* with an OR of 1.92, however only two studies were included indicating the need for more research.

Also produced by type 1 T-helper cells is the cytokine TNFA (El-Far, El-Sayed *et al.*, 2009;Liu, Wang *et al.*, 2010). The *TNFA -308G/A* variant remained significantly associated with recurrent miscarriages; pooled OR 1.46 (1.17-1.84), including seven studies. This is in contrast with the meta-analysis performed in 2009 by Medica et al (Medica, Ostojic *et al.*, 2009), which did not find a significant association. Since the publication of the latter meta-analysis, new large studies were published in women with three or more consecutive miscarriages that did find an association between the variant and recurrent miscarriage (Alkhuriji, Alhimaidi *et al.*, 2013;Gupta, Prakash *et al.*, 2012). We were able to include those. The *-1031C/T* variant also remained significantly associated with recurrent miscarriage in our meta-analysis. The variant was investigated in only 2 studies resulting in an OR of 2.64 (95%CI 1.90-3.66). The polymorphisms described above in pro-inflammatory cytokines (in the genes IFNG, IL18 and TNFA) are in line with the hypothesis of a change in the maternal T-helper cell response; in women with recurrent miscarriage towards a persisting Th1- type (Hill, Polgar *et al.*, 1995).

STAT3 belongs to a family of signal transducers and activators of transcription (STAT1-STAT6) of intracellular signalling proteins and plays a role in unrestrained growth of human tumours (Bromberg and Darnell, Jr., 2000). Two studies regarding the *rs1053004* variant could be included in the meta-analysis and the polymorphism remained significantly

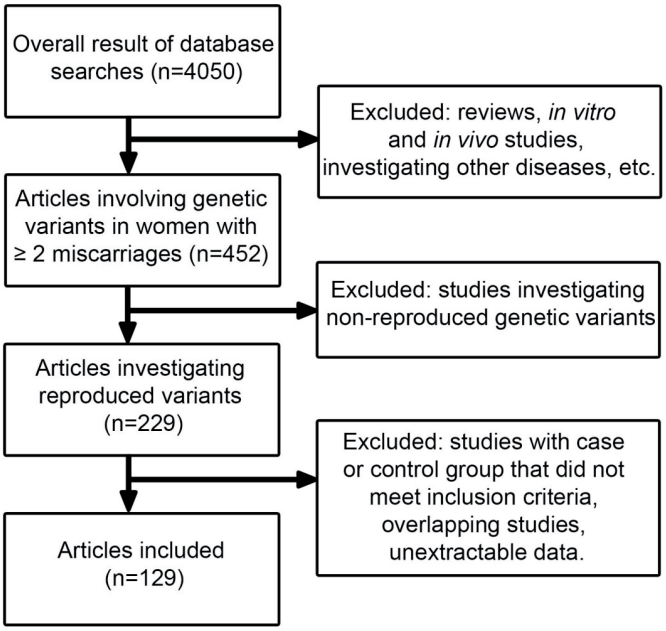


Figure 1. Selection of articles

associated with recurrent miscarriage. Further investigation is needed to clarify the possible mechanisms of the association between the influence of STAT3 on recurrent miscarriage (Garcia, Tirado-Gonzalez *et al.*, 2007). It is hypothesized that altered STAT3 activity leads to a local inflammatory state through recruitment of inflammatory cells and up-regulation of proinflammatory cytokines at the placental membrane, leading to accelerated miscarriage (Messoudi, Al-Sulaiti *et al.*, 2013). VEGFA is an endothelial cell specific protein that acts as a mediator of angiogenesis and vasculogenesis (Almawi, Saldanha *et al.*, 2013). VEGFA is essential in establishing a vascular network during early embryo development (Byrne, Bouchier-Hayes *et al.*, 2005). All three included polymorphisms (*VEGFA -583T/C, 936C/T and -1154G/A*) remained significantly associated with recurrent miscarriage after meta-analysis suggesting an important role in the pathogenesis of recurrent miscarriage.

The use of genetic information is expected to play a major role in future personalized medicine (Chan and Ginsburg, 2011). Personalized medicine is a medical model including each person's unique clinical, genetic, genomic, and environmental information to better classify disease, facilitate the development of new targeted therapies and to more accurately determine disease predisposition which will be helpful in disease prevention.

Cardiovascular disease

Recent epidemiological studies suggest a relationship between recurrent miscarriage and a risk for cardiovascular disease later in life (Oliver-Williams, Heydon *et al.*, 2013;Ranthe,

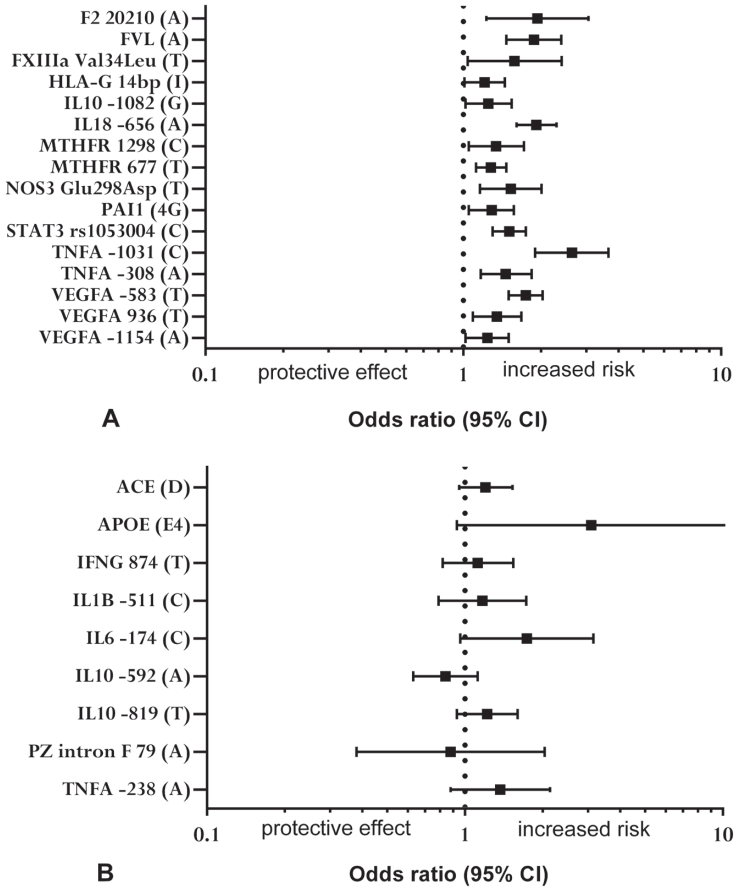
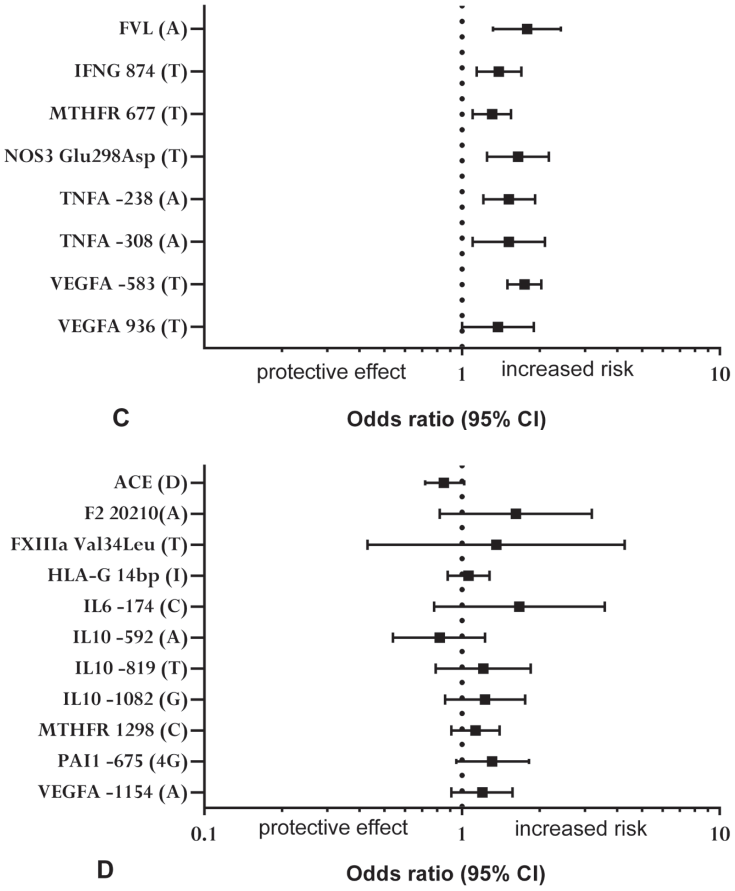


Figure 2. Odds ratio's with 95% confidence intervals for variants that were reproducibly associated with recurrent miscarriage

Andersen *et al.*, 2013). Smith et al found an association between recurrent miscarriage and a family history of ischemic heart disease (Smith, Wood *et al.*, 2011), suggesting a shared genetic predisposition between the two diseases. It is noteworthy to mention that several of the variants that were associated with recurrent miscarriage in this meta-analysis are also identified as risk factors for developing cardiovascular disease. For example *F2 G20210A*, *FVL* and *PAI1 -675 4G/5G* polymorphism were associated with coronary disease in a large meta-analysis (Ye, Liu *et al.*, 2006). In another meta-analysis, *NOS3 Glu298Asp* was found to be significantly associated with ischemic heart disease (Casas, Bautista *et al.*, 2004). *F2 G20210A, FVL, FXIIIa Val34Leu, MTHFR A1298C, MTHFR*



Legend

A. All reproduced genetic variants that were significantly associated with recurrent miscarriage after meta-analysis

B. All reproduced genetic variants that were not significantly associated with recurrent miscarriage after meta-analysis

C. Subgroup analysis: All reproduced genetic variants that were significantly associated with three or more consecutive miscarriages after meta-analysis

D. Subgroup analysis: All reproduced genetic variants that were not significantly associated with three or more consecutive miscarriages after meta-analysis

C677T, NOS3 Glu298/Asp and *PAI1 -675 4G/5G* were associated with ischemic stroke in another meta-analysis (Casas, Hingorani *et al.*, 2004;Kang, Wu *et al.*, 2014).

In summary our study found 16 genetic variants that are associated with recurrent miscarriage. This meta-analysis gives a comprehensive overview of the involved pathways; coagulation and fibrinolysis, immunology and inflammation and angiogenesis. Further studies are needed to investigate the mechanisms of how these genetic variants affect the risk of developing recurrent miscarriages. Additionally, this meta-analysis suggests that recurrent miscarriage and cardiovascular disease share genetic risk factors.

Table 1. Random effect meta-analysis of reproduced genes for recurrent miscarriage

Variant		Minor Allele	Studies(n)	Cases(n)	Controls(n)	OR(95% CI)	I^2 (%)	Egger test P value
ACE I/D	rs1799752	D	11	1928	1170	1.20 (0.95, 1.53)	76.8	0.52
APOE	rs429358	E4	5	969	532	3.09 (0.93,10.24)	84.3	--
F2 G20210A	rs1799963	A	26	3989	3424	1.94 (1.23,3.06)	49.0	0.96
FVL	rs6025	A	38	6025	5246	1.88 (1.47,2.40)	47.2	0.10
FXIIIa Val34Leu	rs5985	T	8	836	568	1.58 (1.04,2.41)	71.3	--
HLA-G 14bp I/D	rs1704	I	11	1449	1362	1.21 (1.01, 1.45)	57.0	0.36
IFNG 874A/T	rs2430561	T	5	596	620	1.12 (0.82, 1.54)	67.6	--
IL1B -511C/T	rs16944	C	4	808	527	1.17 (0.79, 1.73)	81.7	--
IL6 -174G/C	rs1800795	C	4	616	715	1.74 (0.96, 3.15)	87.1	0.34
IL10 -592C/A	rs1800872	A	7	879	1062	0.84 (0.63, 1.12)	73.2	--
IL10 -819C/T	rs1800871	T	4	720	841	1.22 (0.93, 1.60)	67.8	--
IL10 -1082A/G	rs1800896	G	6	1193	1144	1.25 (1.02, 1.54)	52.6	--
IL18 -656C/A	rs1946519	A	2	517	524	1.92 (1.61, 2.30)	0.0	--
MTHFR A1298C	rs1801131	C	14	2289	1758	1.34 (1.05, 1.72)	79.3	0.67
MTHFR C677T	rs1801133	T	32	4146	4573	1.28 (1.12, 1.47)	63.4	0.44
NOS3 Glu298Asp	rs1799983	T	8	1574	1352	1.53 (1.16, 2.01)	72.0	--
PAI1 -675 4G/5G	rs1799889	4G	14	2759	2091	1.29 (1.05, 1.57)	78.8	0.56
PZ intron F G79A	rs3024718	A	4	420	433	0.88 (0.38, 2.04)	87.2	--
STAT3	rs1053004	C	2	670	751	1.51 (1.30, 1.75)	0.0	--
TNFA -238G/A	rs361525	A	5	1058	1201	1.37 (0.88, 2.14)	64.3	--
TNFA -308G/A	rs1800629	A	7	1276	1463	1.46 (1.17, 1.84)	28.5	--
TNFA -1031C/T	rs1799964	C	2	561	484	2.64 (1.90, 3.66)	23.8	--
VEGFA -583T/C	rs3025020	T	3	696	785	1.75 (1.50, 2.03)	0.0	--
VEGFA 936C/T	rs3025039	T	9	1509	1640	1.35 (1.09, 1.68)	50.6	--
VEGFA -1154G/A	rs1570360	A	10	1879	1972	1.24 (1.02, 1.50)	66.1	0.41

Table 2. Subgroup analysis: Random effect meta–analysis of reproduced genes for three or more consecutive miscarriages

Variant	Minor Allele	Studies(n)	Cases(n)	Controls(n)	OR(95% CI)	I^2 (%)	Egger test P value
ACE I/D	D	4	544	593	0.85 (0.72, 1.02)	0.0	--
F2 G20210A	A	14	2314	1911	1.62 (0.82, 3.19)	51.1	0.25
FVL	A	21	3518	3156	1.79 (1.32, 2.42)	34.2	0.10
FXIIIa Val34Leu	T	3	272	268	1.36 (0.43, 4.28)	90.8	--
HLA-G 14bp I/D	I	4	700	405	1.06 (0.88, 1.28)	5.6	--
IFNG 874A/T	T	3	372	485	1.39 (1.14, 1.70)	0.0	--
IL6 -174G/C	C	3	474	537	1.67 (0.78, 3.58)	90.7	--
IL10 -592C/A	A	4	482	601	0.82 (0.54, 1.23)	77.7	--
IL10 -819C/T	T	3	424	536	1.21 (0.79, 1.85)	78.5	--
IL10 -1082A/G	G	3	412	534	1.23 (0.86, 1.76)	68.0	--
MTHFR A1298C	C	7	1019	1106	1.13 (0.91, 1.40)	54.8	--
MTHFR C677T	T	19	1991	2944	1.31 (1.10, 1.55)	60.0	0.31
NOS3 Glu298Asp	T	7	1429	1217	1.65 (1.25, 2.17)	67.3	--
PAI1 -675 4G/5G	4G	7	1042	1377	1.31 (0.95, 1.82)	84.3	--
TNFA -238G/A	A	3	569	813	1.52 (1.21, 1.92)	0.0	--
TNFA -308G/A	A	4	737	1025	1.52 (1.10, 2.10)	53.5	--
VEGFA -583T/C	T	3	696	785	1.75 (1.50, 2.03)	0.0	--
VEGFA 936C/T	T	5	827	901	1.38 (1.00, 1.90)	62.2	--
VEGFA -1154G/A	A	5	1114	1110	1.20 (0.91, 1.57)	71.3	--

Table 3. References of the included articles, per genetic variant

Variant		
ACE I/D		(Al Sallout and Sharif, 2010;Bagheri, Abdi, I *et al.*, 2010;Buchholz, Lohse *et al.*, 2003;Choi, Kwon *et al.*, 2011;Dossenbach-Glaninger, van *et al.*, 2008b;Fatini, Gensini *et al.*, 2000;Kim, Choi *et al.*, 2014;Ozdemir, Yenicesu *et al.*, 2012;Poursadegh, Chaparzadeh *et al.*, 2013;Vettriselvi, Vijayalakshmi *et al.*, 2008;Yenicesu, Cetin *et al.*, 2010)
	Subgroup analysis	(Al Sallout and Sharif, 2010;Bagheri, Abdi, I *et al.*, 2010;Choi, Kwon *et al.*, 2011;Kim, Choi *et al.*, 2014)
APOE		(Agarwal, Parveen *et al.*, 2010;Asgari, Akbari *et al.*, 2013;Korkmazer, Ustunyurt *et al.*, 2013;Ozdemir, Yenicesu *et al.*, 2012;Poursadegh, Farajzadeh *et al.*, 2014)
F2 G20210A		(Abu-Asab, Ayesh *et al.*, 2011;Altintas, Pasa *et al.*, 2007;Bagheri, Rad *et al.*, 2011a;Buchholz, Lohse *et al.*, 2003;Carp, Salomon *et al.*, 2002;Dossenbach-Glaninger, van *et al.*, 2003;Dutra, Fraga *et al.*, 2014;Finan, Tamim *et al.*, 2002;Guerra-Shinohara, Bertinato *et al.*, 2012;Hohlagschwandtner, Unfried *et al.*, 2003;Ivanov, Komsa-Penkova *et al.*, 2009;Karata, Aydin *et al.*, 2012;Lino, Traina *et al.*, 2014;Mitic, Kovac *et al.*, 2010;Mohamed, El Moaty *et al.*, 2010;Mougiou, Androutsopoulos *et al.*, 2008;Mtiraoui, Borgi *et al.*, 2004;Parand, Zolghadri *et al.*, 2013;Parveen, Shukla *et al.*, 2013;Pihusch, Buchholz *et al.*, 2001;Poursadegh, Chaparzadeh *et al.*, 2013;Serrano, Lima *et al.*, 2011;Sotiriadis, Vartholomatos *et al.*, 2007;Sottilotta, Oriana *et al.*, 2006;Yenicesu, Cetin *et al.*, 2010;Yildiz, Yavuzcan *et al.*, 2012

Variant		
	Subgroup analysis	(Altintas, Pasa *et al.*, 2007;Bagheri, Rad *et al.*, 2011a;Guerra-Shinohara, Bertinato *et al.*, 2012;Hohlagschwandtner, Unfried *et al.*, 2003;Karata, Aydin *et al.*, 2012;Lino, Traina *et al.*, 2014;Mitic, Kovac *et al.*, 2010;Mohamed, El Moaty *et al.*, 2010;Mougiou, Androutsopoulos *et al.*, 2008;Mtiraoui, Borgi *et al.*, 2004;Parand, Zolghadri *et al.*, 2013;Parveen, Shukla *et al.*, 2013;Serrano, Lima *et al.*, 2011;Yildiz, Yavuzcan *et al.*, 2012)
FVL		(Abu-Asab, Ayesh *et al.*, 2011;Altintas, Pasa *et al.*, 2007;Bagheri, Rad *et al.*, 2011a;Biswas, Choudhry *et al.*, 2008;Buchholz, Lohse *et al.*, 2003;Carp, Salomon *et al.*, 2002;Dizon-Townson, Kinney *et al.*, 1997;Dossenbach-Glaninger, van *et al.*, 2003;Dutra, Fraga *et al.*, 2014;Eroglu, Yeniel *et al.*, 2006;Finan, Tamim *et al.*, 2002;Guerra-Shinohara, Bertinato *et al.*, 2012;Hashimoto, Shizusawa *et al.*, 1999;Hohlagschwandtner, Unfried *et al.*, 2003;Ivanov, Komsa-Penkova *et al.*, 2009;Karata, Aydin *et al.*, 2012;Kaur, Puri *et al.*, 2013;Kobashi, Kato *et al.*, 2005;Lino, Traina *et al.*, 2014;Mahjoub, Mtiraoui *et al.*, 2005;Mitic, Kovac *et al.*, 2010;Mohamed, El Moaty *et al.*, 2010;Mougiou, Androutsopoulos *et al.*, 2008;Mtiraoui, Borgi *et al.*, 2004;Mukhopadhyay, Saraswathy *et al.*, 2009;Ozdemir, Yenicesu *et al.*, 2012;Parand, Zolghadri *et al.*, 2013;Parveen, Shukla *et al.*, 2013;Pihusch, Buchholz *et al.*, 2001;Poursadegh, Chaparzadeh *et al.*, 2013;Rai, Shlebak *et al.*, 2001;Serrano, Lima *et al.*, 2011;Sotiriadis, Vartholomatos *et al.*, 2007;Sottilotta, Oriana *et al.*, 2006;Yenicesu, Cetin *et al.*, 2010;Yildiz, Yavuzcan *et al.*, 2012;Younis, 2000;Yusoff, Abdullah *et al.*, 2002)
	Subgroup analysis	(Altintas, Pasa *et al.*, 2007;Bagheri, Rad *et al.*, 2011a;Dizon-Townson, Kinney *et al.*, 1997;Eroglu, Yeniel *et al.*, 2006;Guerra-Shinohara, Bertinato *et al.*, 2012;Hashimoto, Shizusawa *et al.*, 1999;Hohlagschwandtner, Unfried *et al.*, 2003;Karata, Aydin *et al.*, 2012;Kaur, Puri *et al.*, 2013;Kobashi, Kato *et al.*, 2005;Lino, Traina *et al.*, 2014;Mahjoub, Mtiraoui *et al.*, 2005;Mitic, Kovac *et al.*, 2010;Mohamed, El Moaty *et al.*, 2010;Mougiou, Androutsopoulos *et al.*, 2008;Mtiraoui, Borgi *et al.*, 2004;Parand, Zolghadri *et al.*, 2013;Parveen, Shukla *et al.*, 2013;Rai, Shlebak *et al.*, 2001;Serrano, Lima *et al.*, 2011;Yildiz, Yavuzcan *et al.*, 2012).
FXIIIa Val34Leu		(Bagheri, Rad *et al.*, 2011b;Dossenbach-Glaninger, van *et al.*, 2003;Elmahgoub, Afify *et al.*, 2014;Jeddi-Tehrani, Torabi *et al.*, 2010;Lino, Traina *et al.*, 2014;Lopez, Vivenes *et al.*, 2006;Poursadegh, Chaparzadeh *et al.*, 2013;Yenicesu, Cetin *et al.*, 2010)
	Subgroup analysis	(Bagheri, Rad *et al.*, 2011b;Elmahgoub, Afify *et al.*, 2014;Lino, Traina *et al.*, 2014;Lopez, Vivenes *et al.*, 2006)
HLA-G 14bp I/D		(Afkhami, Shekari *et al.*, 2014;Aruna, Sirisha *et al.*, 2011;Berger, Hogge *et al.*, 2010;Christiansen, Kolte *et al.*, 2012;Shankarkumar, Shankarkumar *et al.*, 2011;Suryanarayana, Rao *et al.*, 2008;Tripathi, Abbas *et al.*, 2004;Vargas, Sarturi *et al.*, 2011;Xue, Yang *et al.*, 2007;Yan, Lin *et al.*, 2006;Zhu, Huo *et al.*, 2010)
	Subgroup analysis	(Christiansen, Kolte *et al.*, 2012;Suryanarayana, Rao *et al.*, 2008;Tripathi, Abbas *et al.*, 2004;Vargas, Sarturi *et al.*, 2011).
IFNG 874A/T		(Bompeixe, Carvalho Santos *et al.*, 2012;Kamali-Sarvestani, Zolghadri *et al.*, 2005;Parveen, Shukla *et al.*, 2012;Prigoshin, Tambutti *et al.*, 2004;Zastavna, Sosnina *et al.*, 2014)
	Subgroup analysis	(Kamali-Sarvestani, Zolghadri *et al.*, 2005;Parveen, Shukla *et al.*, 2012;Prigoshin, Tambutti *et al.*, 2004)
IL1B -511C/T		(Hefler, Tempfer *et al.*, 2002;Kim, Lee *et al.*, 2014;Ma, Xu *et al.*, 2012;Wang, Yunis *et al.*, 2002)
IL6 -174G/C		(Demirturk, Ates *et al.*, 2014;Parveen, Shukla *et al.*, 2012;Unfried, Bocskor *et al.*, 2003;von, Bompeixe *et al.*, 2005)
	Subgroup analysis	(Demirturk, Ates *et al.*, 2014;Parveen, Shukla *et al.*, 2012;Unfried, Bocskor *et al.*, 2003)

Variant		
IL10 -592C/A		(Alkhuriji, Alhimaidi *et al.*, 2013;Bahadori, Zarei *et al.*, 2014;Kamali-Sarvestani, Zolghadri *et al.*, 2005;Kaur and Kaur, 2011;Parveen, Shukla *et al.*, 2012;Qaddourah, Magdoud *et al.*, 2014;Zastavna, Sosnina *et al.*, 2014)
	Subgroup analysis	(Alkhuriji, Alhimaidi *et al.*, 2013;Bahadori, Zarei *et al.*, 2014;Kamali-Sarvestani, Zolghadri *et al.*, 2005;Parveen, Shukla *et al.*, 2012)
IL10 -819C/T		(Bahadori, Zarei *et al.*, 2014;Kamali-Sarvestani, Zolghadri *et al.*, 2005;Parveen, Shukla *et al.*, 2012;Qaddourah, Magdoud *et al.*, 2014)
	Subgroup analysis	(Bahadori, Zarei *et al.*, 2014;Kamali-Sarvestani, Zolghadri *et al.*, 2005;Parveen, Shukla *et al.*, 2012)
IL10 -1082A/G		(Bahadori, Zarei *et al.*, 2014;Kamali-Sarvestani, Zolghadri *et al.*, 2005;Kim, Lee *et al.*, 2014;Parveen, Shukla *et al.*, 2012;Qaddourah, Magdoud *et al.*, 2014;Zastavna, Sosnina *et al.*, 2014)
	Subgroup analysis	(Bahadori, Zarei *et al.*, 2014;Kamali-Sarvestani, Zolghadri *et al.*, 2005;Parveen, Shukla *et al.*, 2012)
IL18 -656C/A		(Al-Khateeb, Sater *et al.*, 2011;Messaoudi, Dandana *et al.*, 2012)
MTHFR A1298C		(Bae, Choi *et al.*, 2009;Dossenbach-Glaninger, van *et al.*, 2003;Hohlagschwandtner, Unfried *et al.*, 2003;Jeddi-Tehrani, Torabi *et al.*, 2011;Lino, Traina *et al.*, 2014;Mtiraoui, Zammiti *et al.*, 2006;Nair, Khanna *et al.*, 2012;Ozdemir, Yenicesu *et al.*, 2012;Parveen, Tuteja *et al.*, 2013;Poursadegh, Chaparzadeh *et al.*, 2012;Seremak-Mrozikiewicz, Drews *et al.*, 2010;Sotiriadis, Vartholomatos *et al.*, 2007;Yenicesu, Cetin *et al.*, 2010;Yousefian, Kardi *et al.*, 2014)
	Subgroup analysis	(Bae, Choi *et al.*, 2009;Hohlagschwandtner, Unfried *et al.*, 2003;Lino, Traina *et al.*, 2014;Mtiraoui, Zammiti *et al.*, 2006;Parveen, Tuteja *et al.*, 2013;Seremak-Mrozikiewicz, Drews *et al.*, 2010;Yousefian, Kardi *et al.*, 2014)
MTHFR C677T		(Abu-Asab, Ayesh *et al.*, 2011;Bae, Choi *et al.*, 2009;Biswas, Choudhry *et al.*, 2008;Buchholz, Lohse *et al.*, 2003;Creus, Deulofeu *et al.*, 2012;Dossenbach-Glaninger, van *et al.*, 2003;Dutra, Fraga *et al.*, 2014;Eroglu, Yeniel *et al.*, 2006;Hohlagschwandtner, Unfried *et al.*, 2003;Jeddi-Tehrani, Torabi *et al.*, 2011;Karata, Aydin *et al.*, 2012;Kaur, Puri *et al.*, 2013;Kobashi, Kato *et al.*, 2005;Lino, Traina *et al.*, 2014;Mitic, Kovac *et al.*, 2010;Mohamed, El Moaty *et al.*, 2010;Mtiraoui, Zammiti *et al.*, 2006;Nair, Khanna *et al.*, 2012;Ozdemir, Yenicesu *et al.*, 2012;Park, Han *et al.*, 2011;Parveen, Tuteja *et al.*, 2013;Pihusch, Buchholz *et al.*, 2001;Poursadegh, Chaparzadeh *et al.*, 2012;Puri, Kaur *et al.*, 2013;Seremak-Mrozikiewicz, Drews *et al.*, 2010;Sotiriadis, Vartholomatos *et al.*, 2007;Unfried, Griesmacher *et al.*, 2002;Vettriselvi, Vijayalakshmi *et al.*, 2008;Yenicesu, Cetin *et al.*, 2010;Yildiz, Yavuzcan *et al.*, 2012;Yousefian, Kardi *et al.*, 2014)
	Subgroup analysis	(Bae, Choi *et al.*, 2009;Creus, Deulofeu *et al.*, 2012;Eroglu, Yeniel *et al.*, 2006;Hohlagschwandtner, Unfried *et al.*, 2003;Karata, Aydin *et al.*, 2012;Kaur, Puri *et al.*, 2013;Kobashi, Kato *et al.*, 2005;Lino, Traina *et al.*, 2014;Mitic, Kovac *et al.*, 2010;Mohamed, El Moaty *et al.*, 2010;Mtiraoui, Zammiti *et al.*, 2006;Nair, Khanna *et al.*, 2012;Parveen, Tuteja *et al.*, 2013;Poursadegh, Chaparzadeh *et al.*, 2012;Puri, Kaur *et al.*, 2013;Seremak-Mrozikiewicz, Drews *et al.*, 2010;Unfried, Griesmacher *et al.*, 2002;Yildiz, Yavuzcan *et al.*, 2012;Yousefian, Kardi *et al.*, 2014)
NOS3 Glu298Asp		(Almawi, Guarino *et al.*, 2013;Dutra, Fraga *et al.*, 2014;Hefler, Tempfer *et al.*, 2002;Karvela, Papadopoulou *et al.*, 2008;Luo, Li *et al.*, 2013;Parveen, Faridi *et al.*, 2011;Shin, Lee *et al.*, 2010;Suryanarayana, Rao *et al.*, 2006)
	Subgroup analysis	(Almawi, Guarino *et al.*, 2013;Hefler, Tempfer *et al.*, 2002;Karvela, Papadopoulou *et al.*, 2008;Luo, Li *et al.*, 2013;Parveen, Faridi *et al.*, 2011;Shin, Lee *et al.*, 2010;Suryanarayana, Rao *et al.*, 2006)

Variant		
PAI1 -675 4G/5G		(Al Sallout and Sharif, 2010;Buchholz, Lohse *et al.*, 2003;Dossenbach-Glaninger, van *et al.*, 2003;Elmahgoub, Afify *et al.*, 2014;Jeddi-Tehrani, Torabi *et al.*, 2011;Jeon, Kim *et al.*, 2013;Kim, Choi *et al.*, 2014;Lino, Traina *et al.*, 2014;Magdoud, Herbepin *et al.*, 2013;Ozdemir, Yenicesu *et al.*, 2012;Parveen, Tuteja *et al.*, 2013;Poursadegh, Chaparzadeh *et al.*, 2013;Subrt, Ulcova-Gallova *et al.*, 2013;Yenicesu, Cetin *et al.*, 2010)
	Subgroup analysis	(Al Sallout and Sharif, 2010;Elmahgoub, Afify *et al.*, 2014;Kim, Choi *et al.*, 2014;Lino, Traina *et al.*, 2014;Magdoud, Herbepin *et al.*, 2013;Parveen, Tuteja *et al.*, 2013;Subrt, Ulcova-Gallova *et al.*, 2013)
PZ intron F G79A		(Al-Shaikh, Sater *et al.*, 2013;Dossenbach-Glaninger, van *et al.*, 2008a;El-Hamid and El-Khayat, 2011;Topalidou, Effraimidou *et al.*, 2009)
STAT3		(Finan, Mustafa *et al.*, 2010;Messoudi, Al-Sulaiti *et al.*, 2013)
TNFA -238G/A		(Alkhuriji, Alhimaidi *et al.*, 2013;Finan, Al-Irhayim *et al.*, 2010;Gupta, Prakash *et al.*, 2012;Lee, Jeon *et al.*, 2013;Liu, Wang *et al.*, 2010)
	Subgroup analysis	(Alkhuriji, Alhimaidi *et al.*, 2013;Finan, Al-Irhayim *et al.*, 2010;Gupta, Prakash *et al.*, 2012)
TNFA -308G/A		(Alkhuriji, Alhimaidi *et al.*, 2013;Finan, Al-Irhayim *et al.*, 2010;Gupta, Prakash *et al.*, 2012;Kaur and Kaur, 2011;Lee, Jeon *et al.*, 2013;Liu, Wang *et al.*, 2010;Pietrowski, Bettendorf *et al.*, 2004)
	Subgroup analysis	(Alkhuriji, Alhimaidi *et al.*, 2013;Finan, Al-Irhayim *et al.*, 2010;Gupta, Prakash *et al.*, 2012;Pietrowski, Bettendorf *et al.*, 2004)
TNFA -1031C/T		(Finan, Al-Irhayim *et al.*, 2010;Lee, Jeon *et al.*, 2013)
VEGFA -583T/C		(Al-Khateeb, Mustafa *et al.*, 2011;Almawi, Saldanha *et al.*, 2013;Li, Donghong *et al.*, 2013)
	Subgroup analysis	(Al-Khateeb, Mustafa *et al.*, 2011;Almawi, Saldanha *et al.*, 2013;Li, Donghong *et al.*, 2013)
VEGFA 936C/T		(Aggarwal, Parveen *et al.*, 2011;Almawi, Saldanha *et al.*, 2013;Eller, Branch *et al.*, 2011;Lee, Hong *et al.*, 2010;Li, Donghong *et al.*, 2013;Magdoud, Dendana *et al.*, 2012;Papazoglou, Galazios *et al.*, 2005;Samli, Demir *et al.*, 2012;Traina, Daher *et al.*, 2011) (Aggarwal, Parveen *et al.*, 2011;Almawi, Saldanha *et al.*, 2013;Li, Donghong *et al.*, 2013;Magdoud, Dendana *et al.*, 2012;Papazoglou, Galazios *et al.*, 2005;Traina, Daher *et al.*, 2011)
VEGFA -1154G/A		(Aggarwal, Parveen *et al.*, 2011;Almawi, Saldanha *et al.*, 2013;Eller, Branch *et al.*, 2011;Lee, Hong *et al.*, 2010;Li, Donghong *et al.*, 2013;Magdoud, Dendana *et al.*, 2012;Papazoglou, Galazios *et al.*, 2005;Samli, Demir *et al.*, 2012;Su, Lin *et al.*, 2011;Xing, Yan *et al.*, 2011)
	Subgroup analysis	(Aggarwal, Parveen *et al.*, 2011;Almawi, Saldanha *et al.*, 2013;Li, Donghong *et al.*, 2013;Magdoud, Dendana *et al.*, 2012;Papazoglou, Galazios *et al.*, 2005;Xing, Yan *et al.*, 2011)

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