ORIGINAL ARTICLE: EARLY PREGNANCY

Systematic review and meta-analysis of genetic association studies in idiopathic recurrent spontaneous abortion

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Objectives: 1) To perform the first comprehensive systematic review of genetic association studies (GASs) in idiopathic recurrent spontaneous abortion (IRSA); 2) to analyze studies according to recurrent spontaneous abortion (RSA) definition and selection criteria for patients and control subjects; and 3) to perform meta-analyses for the association of candidate genes with IRSA.
Design: Systematic review and meta-analysis.
Setting: Not applicable.
Patient(s): Couples with IRSA and their spontaneously aborted embryos.
Intervention(s): Summary odds ratios (ORs) were calculated by means of fixed- or random-effects models.
Main Outcome Measure(s): Association of genetic variants with IRSA.
Result(s): The systematic review included 428 case-control studies (1990–2015), which differed substantially regarding RSA definition, clinical evaluation of patients, and selection of control subjects. In women, 472 variants in 187 genes were investigated. Meta-analyses were performed for 36 variants in 16 genes. Association with IRSA defined as three or more spontaneous abortions (SAs) was detected for 21 variants in genes involved in immune response (IFNG, IL10, KIR2DS2, KIR2DS3, KIR2DS4, MBL, TNF), coagulation (F2, F5, PAI-1, PROZ), metabolism (GSTT1, MTHFR), and angiogenesis (NOS3, VEGFA). However, ORs were modest (0.51–2.37), with moderate or weak epidemiologic credibility. Minor differences in summary ORs were detected between IRSA defined as two or more and as three or more SAs. Male partners were included in 12.1% of studies, and one study included spontaneously aborted embryos.
Conclusion(s): Candidate gene studies show moderate associations with IRSA. Owing to large differences in RSA definition and selection criteria for participants, consensus is needed. Future GASs should include both partners and spontaneously aborted embryos. Genome-wide association studies and large-scale replications of identified associations are recommended. (Fertil Steril® 2016;■: ■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Candidate gene, evidence-based medicine, genetic polymorphism, meta-analysis, miscarriage

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Recurrent spontaneous abortion (RSA), occurring in 1% of fertile couples, is a pregnancy complication with a heterogeneous nomenclature (recurrent pregnancy loss, recurrent miscarriage, habitual abortion) and definition. According to guidelines for the investigation and treatment of couples with RSA, the condition is defined by the European Society for Human Reproduction and Embryology (ESHRE) and Royal College of Obstetricians and Gynaecologists (RCOG) as three or more consecutive spontaneous abortions (SAs), whereas the American Society for Reproductive Medicine (ASRM) defines it as two or more SAs, although it recommends that only couples with three or more SAs be included in epidemiologic studies (1–3). In all three guidelines, known causes of RSA include antiphospholipid syndrome

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(APS) and uterine anatomic anomalies (UAAs) in women, and chromosome abnormalities (CAs) in either partner. Although various hypotheses have been tested, causative factors in the remaining couples (~50%) have not been identified.

The assumption for a genetic predisposition to idiopathic RSA (IRSA) is based on three observations: 1) siblings of patients with IRSA exhibit a higher frequency of SA than population control subjects (4–6); 2) the risk of SA increases with their number (7, 8); and 3) SAs in couples with IRSA recur at the same gestational age (~90% before 12 weeks of gestation) (9). Numerous genetic factors have been tested, including DNA methylation, skewed X chromosome inactivation, chromosome heteromorphisms, sperm DNA fragmentation, and genetic variation, but none has been confirmed unanimously as a major risk factor for IRSA. Genetic association studies (GASs) constitute an especially large amount of scientific papers published in this field and were mostly designed as hypothesis-based candidate gene studies performed in unrelated subjects (10). However, as our group emphasized previously, comparative analyses are complicated owing to large differences between studies regarding the definition of RSA (minimal number, order, and gestational age of SAs), diagnostic procedures performed in patients to exclude the known causes of RSA, and definition of the control group (11, 12). Furthermore, similarly to limitations of GASs in other common diseases, results are often contradictory, not replicated, and/or based on a small number of participants (13). In addition, certain published qualitative and qualitative syntheses show limitations. For example, the criteria for evaluation and inclusion of studies, particularly meta-analyses, are seldom based on professional guidelines for the evaluation of couples with RSA, and the process of study selection is often not conducted in accordance with the proposed criteria (11, 12).

Therefore, to address the current status in the field and contribute to an improved understanding of the role of genetic variation in IRSA, we evaluated the evidence for the association of various candidate genes with IRSA in couples and their offspring through the following specific objectives: 1) to perform a comprehensive systematic review of all GASs in the English language analyzing the association between genetic variants (polymorphisms and mutations) and IRSA; 2) to analyze studies according to IRSA definition and selection criteria for patients and control subjects; 3) to perform meta-analyses and compare summary estimates for each genetic variant among three categories of studies: minimum (all studies with genotype frequencies reported), medium (studies defining IRSA as two or more SAs), and full (studies defining IRSA as three or more SAs) criteria, with other rigorous selection criteria applied for the latter two categories.

MATERIALS AND METHODS

Search Strategy

A systematic review of the literature was conducted with the use of the Pubmed and Scopus electronic databases, which were searched for publications on the association between genetic variants (polymorphisms and mutations) and IRSA from January 1, 1990, to January 1, 2015 (25 years). The following key words were used: "recurrent pregnancy loss," "recurrent miscarriage" or "recurrent spontaneous abortion" in combination with "gene mutation" or "polymorphism." Because Pubmed and Scopus are the medical databases with the best coverage (14, 15), references of retrieved articles were not additionally hand searched. The search for publications was performed independently by two authors, and all retrieved articles were compared to avoid duplication. Any disagreements were discussed and resolved with consensus. Systematic review and meta-analyses were performed in accordance with PRISMA (Preferred Reporting Items of Systematic Reviews and Meta-analyses) guidelines. Considering that this study was a systematic review with meta-analyses, an Institutional Review Board approval was not required.

Study Selection

The objective was to identify case-control studies on the association between genetic variants (polymorphisms and mutations) and IRSA with the use of the following exclusion criteria: non–case-control studies (reviews, case reports, meta-analyses, cohort studies, book chapters, etc.), studies performed in patients with RSA of known cause or patients with IRSA in combination with other disorders (e.g., hydatiform mole) or patients with IRSA tested for other genetic factors (copy number variations, X-chromosome inactivation, epigenetic modifications, mitochondrial DNA variants, genome-wide association studies, Y-chromosome microdeletions), and studies not related to IRSA (other disorders). Language restriction was applied and only reports in the English language were taken into consideration. Congress abstracts were included if results did not overlap with those published in original papers.

Meta-analyses

Considering that there are no universal criteria for the definition of RSA, the criteria for inclusion of studies in meta-analyses of individual genetic variants were divided into three categories for comparative analysis of summary estimates:

1. Minimum criteria: Meta-analyses were performed for all retrieved studies in which genotype frequencies were reported, regardless of RSA definition, selection criteria for patients and control subjects, or deviation of genotype frequencies from Hardy-Weinberg equilibrium (HWE) in the control group.
2. Medium criteria: Meta-analyses were performed for studies in which IRSA was defined as two or more SAs (including three or more SAs) and which met the rigorous inclusion criteria described below.
3. Full criteria: Meta-analyses were performed for studies in which IRSA was defined as three or more SAs and which met the rigorous inclusion criteria described below.

The rigorous inclusion criteria for meta-analyses of studies that appertain to medium and full criteria were: 1) case-control study in which genotyping was performed in women and/or men with IRSA and control women and/or men; 2) diagnosis of IRSA based on ESHRE, RCOG, and