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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® 2017;107: 150–9. Copyright ©2016 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).)
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 (APS) and uterine anatomic anomalies

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(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

ASRM guidelines (exclusion of APS in women, karyotyping of both partners for exclusion of CAs, ultrasonography and/or hysteroscopy for exclusion of UAAs) (1–3); 3) control group defined as women/couples with at least one live birth and no SA; 4) all genotype frequencies reported; and 5) no deviation of genotype frequencies from HWE in the control group. In accordance with previously published large-field synopses in other diseases, comparative analysis for each genetic variant was performed if three or more studies met the full criteria (16).

We introduced the category of minimum criteria because we noticed that in some previously published meta-analyses, all of the retrieved studies in which genotype frequencies were reported were included in quantitative synthesis regardless of the quality of studies (11, 12). Our aim was to objectify the fact that the results of such analyses might be misleading.

If multiple publications from the same author were retrieved, only those with the largest number of participants or where all genotype frequencies were reported were included in meta-analysis. Genetic variants that are not biallelic or have a complex allelic structure (e.g., *HLA*, *ApoE*, *ANXA5*, etc.) were considered for meta-analysis if genotype/allele/haplotype frequencies were uniformly reported between studies.

Data Extraction

The following data were extracted for each study included in the systematic review and meta-analysis: authors, year of publication, population studied, definition of RSA, number of patients and control subjects (women, men, spontaneously aborted conceptuses, children), diagnostic evaluations performed in couples with IRSA (exclusion of APS, CAs, UAAs, and other possible causes), definition of control group, and genetic variants tested. In addition, if a genetic variant was tested in three or more studies, genotype and allele frequencies were extracted and HWE for genotype frequencies in the control group was calculated for each study. If a publication reported genotype frequencies stratified by the number of SAs, all numbers were extracted.

Statistical Analysis

Conformity of genotype frequencies to HWE in the control group was tested with the use of the Simple Hardy-Weinberg Calculator–Court Lab (Washington State University College of Veterinary Medicine). Deviation from HWE was determined by a P value $<.05$.

Meta-analyses were performed with the use of Comprehensive Meta-analysis, version 2.2.064 (Biostat). For each study, individual and summary odds ratios (ORs) and associated 95% confidence intervals (CIs) were calculated under dominant and/or recessive genetic models or special models (e.g., *HLA*, *KIR*, etc.). Associations were confirmed by P values $<.05$. Tests for statistical heterogeneity were performed for each meta-analysis with the use of Cochran Q test. If the P value was $<.05$, a fixed-effects model was applied. Otherwise, random-effects model was applied. Because there was no severe statistical heterogeneity in meta-analyses performed under full criteria, sensitivity analysis was not conducted. Publication bias was evaluated under full criteria with the use of the funnel plot and Egger regression test for associations with P values $<.05$.

Assessment of Cumulative Epidemiologic Evidence

Cumulative epidemiologic evidence for genetic variants under full criteria was assessed with the use of the Venice criteria (17). Epidemiologic credibility for each meta-analysis was graded on three levels: amount of evidence, extent of replication, and protection from bias. The amount of evidence was evaluated by the total number of minor allele copies (MAC) in case and control subjects for each variant (grade A: total number of MAC >1,000; grade B: total number of MAC = 100–1,000; grade C: total number of MAC <100). Extent of replication was evaluated by the amount of statistical heterogeneity as measured by means of I^2 (grade A: I^2 <25%; grade B: I^2 = 25%–50%; grade C: I^2 >50%). Considering that we aimed to select well designed studies for inclusion in the meta-analysis under full criteria (e.g., similar phenotype definition, appropriate genotyping methods, no deviations from HWE), protection from bias was evaluated by the magnitude of association, minor differences in IRSA definition, and population differences. Grade A was assigned when the summary OR was >1.15 with uniform IRSA definitions and similar populations involved in analysis; grade B was assigned when the summary OR was >1.15 but minor differences in IRSA definition were present (e.g., consecutive/nonconsecutive SAs) or different populations were involved in analysis; grade C was given when the summary OR was <1.15 or if there was presence of publication bias. Final categories for the credibility of cumulative epidemiologic evidence were assigned as suggested by Ioannidis et al. (17).

RESULTS

A total of 428 case-control studies in the English language on the association between genetic variants and IRSA were identified through the comprehensive literature searching (Supplemental Fig. 1; Supplemental Figs. 1 and 2, Supplemental Tables 1–6, and Supplemental Appendix 1 are available online at www.fertstert.org). The distribution of these studies in the past 25 years is shown in Supplemental Figure 2, and the numbers of participants tested are presented in Supplemental Table 1. Because of the comprehensiveness of the present systematic review, we present only descriptive data in the tables, and the complete literature list is available in Supplemental Appendix 1.

Definition of Recurrent Spontaneous Abortion

Different definitions of RSA in retrieved studies are presented in Table 1. The definition was evaluated according to the following criteria: number, order, and gestation period of SAs, whether the SAs occurred with the same partner, number of live born children, and other inclusion criteria. We detected huge differences among the studies, with a total of 130 different definitions: 160 studies defined RSA as two or more SAs (37.4%), 227 studies defined RSA as three or more

TABLE 1

Definitions of recurrent spontaneous abortion in retrieved studies in descending order.

		Order				Live birth			
Definition	SAs, n	Consecutive	Nonconsecutive	SAs with same partner	Gestation period	Primary	Secondary	Other	Studies, n
1.	≥2	+							33
2.	≥2								22
3.	≥2	+			<20 WOG				9
4.	≥2				<20 WOG				8
5.	≥2	+				+	+		7
6.	≥2					+	+		7
7.	≥2	+			<12 WOG				7
8.	≥2	+				+			6
9.	≥2					+			6
10.	≥2	+				+	+	SA and SB	5
11.	≥2				1st trimester				4
12.	≥2	+			<25 WOG				3
13.	≥2			+	<24 WOG	+			3
14.	≥2			+	<12 WOG	+			3
15.	≥2	+		+					2
16.	≥2							SA and SB	2
17.	≥2	+			<22 WOG				2
18.	≥2	+			<20 WOG	+			2
19.	≥2	+		+	5–12 WOG	+			2
20.	≥2	+			1st trimester				2
21.	≥2				During pregnancy				2
22.	≥2			+					1
23.	≥2					+	+	Clinical SA	1
24.	≥2	+			<25 WOG	+			1
25.	≥2				<20 WOG	+			1
26.	≥2				<20 WOG	+	+		1
27.	≥2	+			<20 WOG	+	+		1
28.	≥2	+			<20 WOG			<35 YOA	1
29.	≥2	+	+		<20 WOG				1
30.	≥2	+		+	<20 WOG				1
31.	≥2	+		+	<20 WOG	+			1
32.	≥2				<17 WOG				1
33.	≥2	+		+	<17 WOG				1
34.	≥2			+	<17 WOG				1
35.	≥2	+		+	<15 WOG				1
36.	≥2	+		+	<14 WOG				1
37.	≥2			+	<14 WOG	+			1
38.	≥2				<13 WOG	+	+	Clinical SA	1
39.	≥2	+		+	8–12 WOG				1
40.	≥2	+			6–10 WOG				1
41.	≥2	+			1st trimester	+			1
42.	≥2	+			During pregnancy				1
43.	≥2	+			Early pregnancy	+			1
44.	≥2	+				Max 1 live birth			1
45.	≥3								25
46.	≥3					+			20
47.	≥3	+							16
48.	≥3	+		+	<20 WOG				14
49.	≥3	+			<20 WOG				11
50.	≥3			+	<20 WOG				9
51.	≥3				1st trimester	+			8
52.	≥3	+			Early pregnancy	+	+		8
53.	≥3				1st trimester				7
54.	≥3	+			<20 WOG	+			5
55.	≥3	+		+	1st trimester	+	+		5
56.	≥3	+				+			4
57.	≥3	+				+	+		4
58.	≥3	+		+	<24 WOG				4
59.	≥3				<20 WOG				4
60.	≥3	+		+	<20 WOG	+			4
61.	≥3	+		+	1st trimester				4
62.	≥3			+					3
63.	≥3					+	+		3
64.	≥3	+			5–30 WOG				3
65.	≥3				<24 WOG				3

Pereza. Genetic associations with IRSA. Fertil Steril 2016.

TABLE 1

Continued.

Definition	SAs, n	Order		SAs with same partner	Gestation period	Live birth		Other	Studies, n
		Consecutive	Nonconsecutive			Primary	Secondary		
66.	≥3	+			5–21 WOG				3
67.	≥3	+			<13 WOG			2nd trimester, IUFD	3
68.	≥3				<12 WOG	+			3
69.	≥3	+		+	<12 WOG				3
70.	≥3	+			1st trimester				3
71.	≥3	+		+					2
72.	≥3			+		+	+		2
73.	≥3	+		+	<30 WOG				2
74.	≥3	+			<24 WOG				2
75.	≥3	+			<22 WOG	+	+		2
76.	≥3				<20 WOG	Max 1 livebirth			2
77.	≥3				<12 WOG				2
78.	≥3	+			<10 WOG				2
79.	≥3			+	1st trimester				2
80.	≥3			+	1st trimester	+	+		2
81.	≥3				1st or 2nd trimester				2
82.	≥3	+	+						1
83.	≥3	+		+		+	+		1
84.	≥3				<30 WOG				1
85.	≥3	+			<30 WOG	+	+		1
86.	≥3				<28 WOG	+	+		1
87.	≥3				<28 WOG	+	+	+ SB	1
88.	≥3	+			<28 WOG	+	+		1
89.	≥3	+			<28 WOG	+	+	+ SB	1
90.	≥3	+			<25 WOG				1
91.	≥3				<23 WOG				1
92.	≥3			+	<23 WOG				1
93.	≥3	+			<22 WOG				1
94.	≥3			+	<22 WOG				1
95.	≥3	+			5–21 WOG	+	+		1
96.	≥3	+		+	<20 WOG	+	+		1
97.	≥3	+		+	6–20 WOG				1
98.	≥3				10–20 WOG				1
99.	≥3	+		+	<14 WOG				1
100.	≥3	+			5–14 WOG			Clinical SA	1
101.	≥3	+			6–13 WOG				1
102.	≥3			+	<12 WOG				1
103.	≥3	+		+	<12 WOG	+	+		1
104.	≥3	+		+	1st trimester	+			1
105.	≥3	+			1st trimester	+	+		1
106.	≥3				any trimester				1
107.	≥3	+	+		1st or 2nd trimester				1
108.	1–5								1
109.	≥4								1
110.	≥5								1
111.	ND								12

Special criteria

112.	≥2 SAs in 1st trimester or ≥1 late SA	3
113.	≥2 consecutive SAs or 3–6 nonconsecutive early SAs	3
114.	≥2 consecutive SAs or 3–6 nonconsecutive SAs (8–12 WOG)	2
115.	2 consecutive SAs or ≥3 nonconsecutive SAs (8–12 WOG)	2
116.	≥3 consecutive SAs <12 WOG or ≥1 late miscarriage (>12 WOG)	2
117.	≥2 SAs <24 WOG or ≥1 IUFD >24 WOG and max. 1 livebirth	1
118.	≥2 SAs <12 WOG or 1 SA >12 WOG	1
119.	≥2 SAs <10 WOG or ≥1 mid-trimester or 3rd trimester IUFD and/or IUGR	1

Pereza. Genetic associations with IRSA. Fertil Steril 2016.

TABLE 1

Continued.

	Special criteria	
120.	≥2 consecutive SAs ≥21 WOG with the same partner or ≥1 SA >21 WOG	1
121.	≥2 consecutive SAs in 1st trimester or ≥3 SAs in 1st trimester (≥16 WOG)	1
122.	≥3 SAs in 1st trimester or ≥2 SAs in 2nd or 3rd trimester	1
123.	≥3 SAs in any trimester or 1 SA in 2nd or 3rd trimester	1
124.	≥3 consecutive SAs <12 WOG and no late fetal loss or at ≥1 late fetal loss (>12 WOG)	1
125.	≥3 consecutive SAs in 1st trimester or 2 consecutive SAs in 2nd trimester or ≥1 IUFD	1
126.	≥3 consecutive SAs or ≥2 consecutive SAs followed by severe IUGR or placenta abruption, primary/secondary	1
127.	≥3 consecutive primary SAs <10 WOG or 2 consecutive SAs ≥10 WOG	1
128.	≥3 consecutive primary SAs <12 WOG or 2 SAs >12 WOG	1
129.	≥3 consecutive primary/secondary SAs <12 WOG and ≥2 late SAs (>12 WOG)	1
130.	unclear: ≥2 SAs <20 WOG (later in text ≥3 primary SAs)	1

Note: IUFD = intrauterine fetal death; IUGR = intrauterine growth restriction; ND = not defined; SA = spontaneous abortion; SB = stillbirth; WOG = weeks of gestation; YOA = years of age.

Pereza. Genetic associations with IRSA. Fertil Steril 2016.

SAs (53.0%), 29 studies had special criteria for definition (6.8%), and no definition was indicated in 12 studies (2.8%). Within these categories, studies also differed to a great extent regarding other criteria.

Exclusion of Known Causes of Recurrent Spontaneous Abortion

All three known causes of RSA (APS, CA, UAA) were excluded in 220 (51.4%) studies (Table 2). Other diagnostic procedures performed in patients with IRSA, mostly women, were extensive and mainly included testing for hereditary and acquired thrombophilias and endocrine, autoimmune, and metabolic disorders.

Selection Criteria for the Control Group

Selection criteria for the control group are presented in Table 3. We found 23 different combinations of inclusion criteria regarding the number of SAs and live born children and whether there were no other pregnancy complications.

Genetic Variants Investigated in Women

In women with IRSA, 472 variants in 187 genes were investigated, involved mostly in immune response, coagulation, metabolism, angiogenesis, endocrine system, and regulation of vascular function (Supplemental Table 2). In addition, in eight genes the variants tested were not specified, and the results of sequencing for 12 genes were too extensive to be displayed individually or the variants identified were not specified. A total of 305 variants (64.6%) in 142 genes and four unspecified variants in four genes were tested in only one study. A further 131 variants (27.8%) in 79 genes were

TABLE 2

Exclusion of known causes of recurrent spontaneous abortion in retrieved studies.

APS	CA	UAA	Not excluded if positive	Studies, n
+	+	+		220
	+	+		103
ND	ND	ND		37
		+		16
			+ (APS, CA, UAA)	15
	+			15
+		+		9
			+ (APS)	5
			+ (CA)	3
			+ (APS, CA)	2
+				3

Note: APS = antiphospholipid syndrome; CA = chromosome abnormality; ND = not defined; UAA = uterine anatomic anomaly.

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TABLE 3

Selection criteria for the control group of women/couples in retrieved studies.

SA, n	Live birth, n	No other pregnancy complication	Studies, n
0	≥2		104
0	≥1		95
ND	ND		42
0	≥1	+	41
0	≥2	+	37
ND	≥2		19
0	Multipara		17
ND	≥1		17
0	ND		15
ND	Multipara		9
0	Multipara	+	6
ND	ND	+	5
0	ND	+	4
≤1	≥2		4
ND	≥1	+	3
≤1	≥1		2
ND	≥2	+	2
ND	Multipara	+	1
Special criteria			
Women with venous thromboembolism, uteroplacental dysfunction, or atherothrombotic disease and women without SA, venous thromboembolism, uteroplacental dysfunction, or atherothrombotic disease			1
≥1 normal pregnancies resulting in a full-term (>37 wk) live birth, with appropriate weight for gestational age, excluding women with >2 SAs or any late loss and non–English-speaking women			1
Nonpregnant women without obstetrical complications or SA, including women with and without live births			1
≥1 successful pregnancies and without RSA or infertility			1
Parous women without RSA or thrombosis			1

Note: ND = not defined; RSA = recurrent spontaneous abortion; SA = spontaneous abortion.

Pereza. Genetic associations with IRSA. Fertil Steril 2016.

tested in at least two studies, but meta-analysis was not performed because no more than two studies met the full criteria (Supplemental Table 3). Finally, 150 meta-analyses were performed for 36 variants (7.6%) in 16 genes.

Full criteria. Association with IRSA defined as three or more SAs was detected for 21 variants in 13 genes (Table 4; Supplemental Tables 4 and 5). Overall, the genetic variants showed modest effects on IRSA, with ORs ranging from 0.51 to 2.37. None of the associations reached strong epidemiologic credibility. Publication bias was detected for the *MTHFR* C677T single-nucleotide polymorphism under a recessive genetic model ($t = 2.6$; $df = 10$; $P = .028$).

Comparison of summary estimates between categories of criteria. Regardless of the category of criteria, the ORs that reached statistical significance were modest (Table 4; Supplemental Table 4). Nevertheless, several differences emerged when comparing the three categories.

The differences between meta-analyses performed under minimum and full criteria are most obvious regarding statistical heterogeneity, which reached significance in 22 meta-analyses under minimum criteria, but was lost in 11 meta-analyses under full criteria. This was especially noticeable for meta-analyses that included the largest number of studies (e.g., *F2* G20210A, *F5* Leiden, *MTHFR* C677T, *MTHFR* A1298C). Association with IRSA was detected for *GSTT1* null/present variant under full criteria but not under minimum criteria. Conversely, compared with minimum criteria, association with RSA was lost for eight variants under full criteria (*GSTM1* null/present, *HLA-G* I/D 14-bp, *IL10* −1082 G/A, *MTHFR* A1298C, *NOS3* +894 G/T, *PAI-1* 4G/5G, *TNF* −308 G/A, *VEGFA* +936 C/T).

Comparison between medium and full criteria was possible for 18 meta-analyses. Statistical heterogeneity and ORs were similar between these categories. However, under full criteria, *GSST1* null/present, *MTHFR* C677T, and *PAI-1* 4G/5G variants became associated with IRSA, whereas association was lost for *IFNG* +874 A/T variant.

Genetic Variants Investigated in Men and Offspring

Genetic variants investigated in male partners of IRSA women are presented in Supplemental Table 6. A total of 73 variants in 42 genes were investigated in 52/428 studies (12.1%). In addition, tested variants were not specified for five genes, and the results of sequencing of three genes were too extensive or the variants identified were not specified. Meta-analyses were not performed.

Live born children of couples with IRSA were tested for *C4A*, *C4B*, *CFB*, *CGB5*, *HLA* (*A*, *B*, *C*, *DRB*, *DQA*, *DQB*, *DPA*, *DPB*), and *LTA* gene variants in four studies, whereas only the *MTHFR* A1298C variant was tested in spontaneously aborted embryos in one study.

DISCUSSION

We performed the first comprehensive systematic review and meta-analysis of all GASs in IRSA published in the past 25 years (1990–2015) to provide quantitative summary estimates of the effect of genetic variants on the odds for IRSA. We evaluated 428 case-control studies, including the largest number of IRSA couples and control subjects in qualitative and quantitative analyses up to now.

Characteristics of Retrieved Studies

The most important quality determinant of any meta-analysis is the inclusion of well designed original studies with identical or nearly identical selection criteria for patients and control subjects, which minimalizes heterogeneity and biased summary estimates. Although initially our goal was solely to compare the summary estimates among different categories of studies, our systematic review revealed many other issues that need to be addressed in future studies. The most

TABLE 4

Results of meta-analyses for gene variants showing association with idiopathic recurrent spontaneous abortion in women under full criteria.

Gene variation		Minimum criteria					Medium criteria					Full criteria				
Gene	Variant	Studies, n	Participants, n (W_P:W_C)	Genetic model	OR (95% CI); *P* value	I^2; *P* value	Studies, n	Participants, n (W_P:W_C)	OR (95% CI); *P* value	I^2; *P* value	Studies, n	Participants, n (W_P:W_C)	OR (95% CI); *P* value	I^2; *P* value	Venice criteria[a]
F2	G20210A[b]	71/54[c]	6,790:6,327	Dominant AA+AG vs. GG	1.75 (1.31–2.35); .000	33.4; .013	25	3,880:3,718	1.77 (1.29–2.42); .000	11.9; .299	13	2,257:1,993	1.73 (1.10–2.73); .018	0.0; .653	CAB (weak)
F5	Leiden[b]	91/72[c]	10,286:9,132	Dominant AA+AG vs. GG	2.19 (1.76–2.71); .000	58.2; .000	29	5,108:4,000	1.61 (1.32–1.97); .000	33.2; .053	14	1,585:1,712	1.74 (1.26–2.39); .001	3.3; .411	BAB (moderate)
GSTT1	null/present	7	1,016:1,026	Null vs. present gene	1.26 (0.88–1.80); .201	62.0; .015	6	901:866	1.35 (0.90–2.04); .148	64.6; .015	4	593:644	1.65 (1.22–2.23); .001	59.9; .058	BCB (weak)
IFNG	+874 A/T	7	683:787	Dominant TT+AT vs. AA	1.27 (1.02–1.59); .036	32.9; .176	4	342:491	1.47 (1.10–1.96); .009	19.8; .291	3	281:416	1.61 (1.18–2.21); .003	0.0; .425	BAB (moderate)
IL10	−1082 G/A	13/11[c]	1,724:1,739	Recessive GG vs. AG+AA	1.54 (1.12–2.12); .009	55.7; .012	3	549:680	1.95 (1.35–2.80); .000	0.0; 1.000	3	549:680	1.95 (1.35–2.80); .000	0.0; 1.000	BAB (moderate)
KIR	KIR2DS2	5/4	345:388		1.58 (1.16–2.16); .004	0.0; .518	3	272:320	1.68 (1.20–2.37); .003	0.0; .451	3	272:320	1.68 (1.20–2.37); .003	0.0; .451	BAB (moderate)
	KIR2DS3	5/4	345:388		1.70 (1.24–2.33); .001	0.0; .547	3	272:320	1.83 (1.28–2.61); .001	0.0; .540	3	272:320	1.83 (1.28–2.61); .001	0.0; .540	BAB (moderate)
	KIR2DS4	5/4	345:388		0.54 (0.38–0.75); .000	47.0; .129	3	272:320	0.51 (0.36–0.72); .000	36.8; .205	3	272:320	0.51 (0.36–0.72); .000	36.8; .205	BBC (weak)
MBL	−550 H/L −221 X/Y codon 52 codon 54 codon 57	4/3[c]	333:408	Low vs. intermediate + high[d]	1.92 (1.12–3.27); .017	55.5; .106	3	333:408	1.92 (1.12–3.27); .017	55.5; .106	3	282:408	1.96 (1.14–3.38); .015	53.9; .114	BCB (weak)
MTHFR	C677T	79/56[c]	7,097:5,911	Dominant TT+CT vs. CC	1.26 (1.08–1.48); .004	73.0; .000	27	3,413:2,912	1.22 (1.00–1.48); .052	66.8; .000	13	1,685:1,491	1.31 (1.13–1.53); .000	28.8; .155	ABB (moderate)
		79/61[c]	7,354:6,517	Recessive TT vs. CT+CC	1.50 (1.25–1.79); .000	47.2; .000			1.48 (1.14–1.91); .003	43.1; .011			1.67 (1.28–2.19); .000	0.0; .568	AAC (weak)
	A1298C	29/20[c]	2,973:2,618	Recessive CC vs. AC+AA	1.86 (1.31–2.64); .000	46.6; .012	9	1,151:1,277	1.53 (1.09–2.14); .013	36.2; .128	6	838:952	1.55 (1.08–2.23); .017	53.4; .057	ACB (weak)
NOS3	+894 G/T	10	1,984:1,612	Dominant TT+GT vs. GG	1.38 (1.00–1.91); .047	76.0; .000	6	1,108:1,107	1.54 (1.03–2.30); .034	77.0; .001	5	963:972	1.78 (1.25–2.55); .001	64.6; .023	BCB (weak)
PROZ	79 G/A	4	420:433	Recessive AA vs. AG+GG	2.37 (1.01–5.56); .047	0.0; .851	3	380:403	2.37 (1.01–5.56); .047	0.0; .851	3	380:403	2.37 (1.01–5.56); .047	0.0; .851	BAB (moderate)
SERPINE1	4G/5G	28/21[c]	3,722:2,948	Dominant 4G4G+4G5G vs. G5G	1.47 (1.13–1.90); .004	79.1; .000	9	1,538:1,507	1.35 (0.98–1.86); .069	71.2; .001	4	673:903	1.71 (1.09–2.67); .019	71.1; .016	ACB (weak)
TNF	−308 G/A	18/15[c]	2,111:2,294	Dominant AA+AG vs. GG	1.25 (1.07–1.46); .006	30.7; .124	4	628:868	1.47 (1.15–1.89); .002	52.2; .099	4	628:868	1.47 (1.15–1.89); .002	52.2; .099	BCB (weak)
VEGFA	−1154 G/A	12/11[c]	1,929:2,139	Recessive AA vs. AG+GG	1.71 (1.34–2.18); .000	42.0; .069	4	808:1,024	1.53 (1.10–2.15); .013	35.2; .201	3	693:854	1.61 (1.13–2.28); .008	45.5; .159	BBB (moderate)
	−634 G/C	7	1,065:1,469	Recessive CC vs. CG+GG	1.41 (1.14–1.74); .001	0.0; .837	4	673:940	1.55 (1.19–2.02); .001	0.0; .840	4	673:940	1.55 (1.19–2.02); .001	0.0; .840	AAB (moderate)

Note: CI = confidence interval; OR = odds ratio; W_P = women patients; W_C = women control subjects.

[a] The first letter represents the amount of evidence, the second letter the extent of replication, and the third letter protection from bias.

[b] Hardy-Weinberg equilibrium was not calculated, because the mutation is dominant and frequencies of mutated homozygotes and heterozygotes are often shown together.

[c] The number after the slash indicates the number of studies in which genotype frequencies were presented and for which meta-analyses were performed.

[d] Hardy-Weinberg equilibrium was not calculated, because genotypes were grouped into three categories.

Pereza. Genetic associations with IRSA. Fertil Steril 2016.

prominent of these is the selection of patients according to definition of RSA. Despite the existence of professional guidelines, we found an astonishing 130 different combinations of the minimum number, order, and gestational age of SAs, number of live born children, and other criteria necessary for the definition of RSA. The combinations of criteria for inclusion in the control group were also numerous; however, there are no recommendations for the definition of the control group for studies of patients with IRSA. Finally, the mandatory diagnostic procedures for couples with RSA were performed in only one-half of the retrieved studies. At the same time, women/couples were tested for a variety of other disorders even in the absence of clinical indications.

The vast number of different selection criteria for patients with IRSA and their control groups points to huge discrepancies between studies, making comparison difficult and calling for an urgent consensus on RSA. These difficulties were finally most strongly indicated by the small number of studies included in meta-analyses under full criteria.

We would also like to emphasize that in some publications, paragraphs describing the selection of participants were written in such an intricate manner that repeated readings were required. If certain data (e.g., genotype frequencies, selection criteria for patients and control subjects, etc.) was missing or was not presented clearly in the original paper, authors were not contacted, because according to STREGA (Strengthening the Reporting of Genetic Association Studies) recommendations, this information should be reported (18). The lack of such data should prompt future researchers to comply with STREGA recommendations to enhance the transparency of reporting and allow future cumulative synthesis of results.

Association of Genetic Variants with Idiopathic Recurrent Spontaneous Abortion in Women

Out of 472 identified variants, the majority (64.6%) were tested in only one study and an additional 27.8% of variants could not be included in meta-analyses, precluding us from making conclusions on their associations with IRSA. Association with IRSA defined as three or more SAs was detected for 21 variants in 13 genes. Considering their function, these genes are 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 of all associations were modest. In addition, although these systems have been associated with IRSA in theory, the pathophysiologic mechanisms of how specific genetic variants might contribute to SA are largely unexplored.

Considering the ongoing debate on the minimum number of SAs necessary for the diagnosis of RSA (two or more vs. three or more SAs), we performed comparative analysis for each genetic variant included in meta-analysis to evaluate whether there is a difference in summary estimates between women with two or more versus three or more SAs. No major differences in statistical heterogeneity and ORs were observed, although under full criteria, three variants became associated with IRSA, whereas association was lost for one variant.

On the other hand, the differences between meta-analyses performed under minimum and full criteria were evident and multiple. The most obvious difference concerned the association with IRSA, the statistical significance of which changed for nine genetic variants under full criteria. Another obvious difference concerned the decline in statistical heterogeneity under full criteria, which is the consequence of reduction in selection bias. Therefore, only studies with well defined patient and control groups should be included in quantitative synthesis, because the results of studies without transparent selection criteria for participants might be false positive or false negative.

Association of Genetic Variants with Idiopathic Recurrent Spontaneous Abortion in Male Partners and Offspring

Our results show that male partners of women with IRSA are largely underrepresented in GASs (12.1% of studies). Unfortunately, the concept that unsuccessful pregnancy is a female issue is still a prevailing one, which we can also confirm from the repeated inquiries of reviewers on why we chose to include male partners in our studies. The association of studied genetic variants with IRSA in male partners could not be estimated, owing to the low number of studies that met the full criteria for meta-analysis. Furthermore, we found only one study in which spontaneously aborted embryos were investigated. The discouraging statistics on the involvement of male partners and spontaneously aborted embryos in GAS emphasizes the need for a necessary shift in thinking on pregnancy in general.

Study Limitations and Strengths

The limitations of our study originate mostly from the original studies. Owing to different diagnostic criteria, meta-analyses under full criteria were performed on a small number of studies. Subgroup analyses in different populations were therefore not performed, although for meta-analyses in which the largest number of studies were included, populations were mostly white. In addition, owing to the large number of RSA definitions in original studies, we defined RSA only as two or more and three or more SAs under medium and full criteria without taking into consideration the order or gestation period of SAs.

There are also several strengths to this study. The search for literature was comprehensive and systematic. To draw attention to the importance of good design of original studies, quantitative analyses were performed under three categories of criteria (minimum, medium, and full). Regardless of the presence of statistical heterogeneity in meta-analyses under full criteria, which might reflect population differences, selection bias was reduced to a minimum. Homogeneity of the study population was increased by applying rigorous criteria for study selection, which were based on ESHRE, RCOG, and ASRM guidelines. Finally, we objectively present for the first time the differences between studies regarding RSA definition, exclusion of known causes of RSA, and selection criteria for the control group.

Future Research

The present results represent the current situation in the field. We strongly recommend the development of guidelines for GASs in IRSA. Such guidelines already exist for preterm birth (19). Original studies should indicate the order, number, and gestation period of SAs, number of live born children, and whether the SAs occurred with the same partner. In addition, in studies in which RSA is defined as two or more SAs, results for women/couples with three or more SAs should be presented separately. Likewise, we recommend the development of universal professional guidelines for evaluation and treatment of RSA. Researchers should also comply with STREGA recommendations.

Future GASs should focus on the most promising associations and hypothesis-free studies. We identified only two genome-wide association studies in our search, but with different risk loci (6, 20). Repetition of well designed single-study research in which associations of genetic variants with IRSA were detected is also advised. Finally, expression studies are needed to clarify the functional role of genetic variants in IRSA.

CONCLUSION

Candidate gene studies show moderate associations with IRSA in women. Due to large differences between studies regarding the definition of RSA and selection criteria for participants, consensus is urgently needed. Future GAS should include both partners with IRSA and spontaneously aborted embryos. Genome-wide association studies and large-scale replications of identified associations should be performed.

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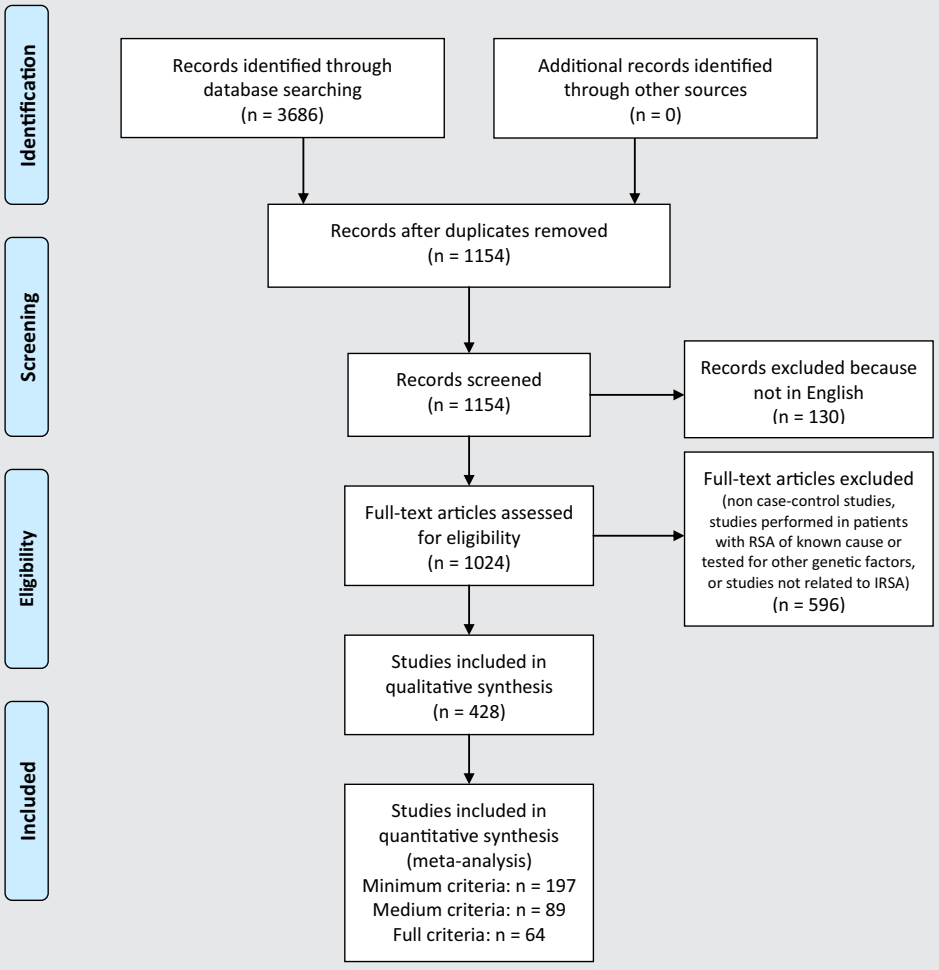
SUPPLEMENTAL FIGURE 1



PRISMA flow diagram, detailing the inclusion and exclusion criteria and the number of studies included and excluded at each step of the literature search.

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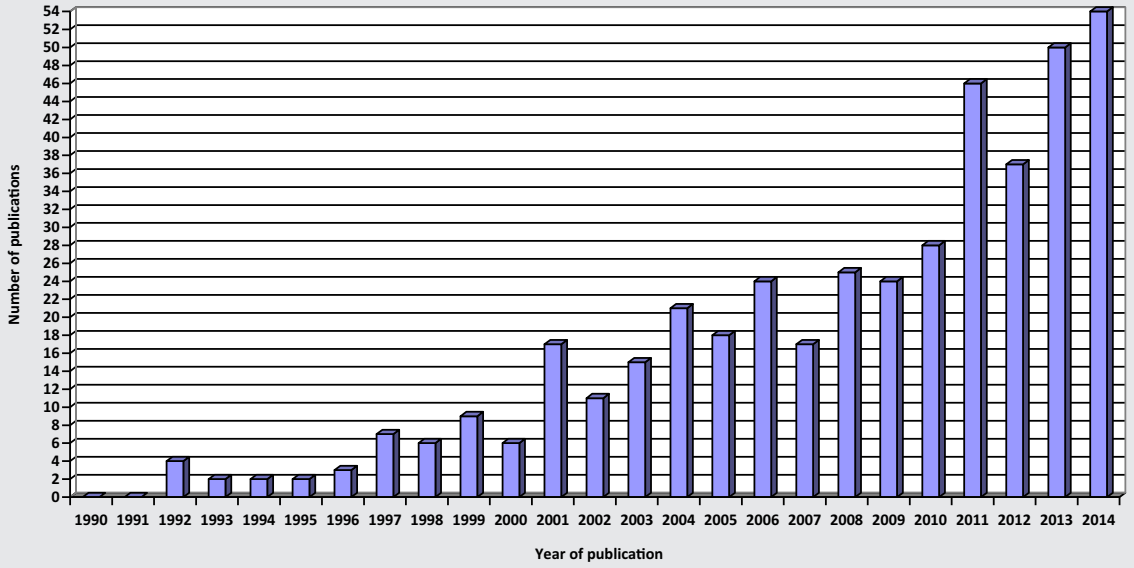
SUPPLEMENTAL FIGURE 2



Distribution of published genetic association studies of idiopathic recurrent spontaneous abortion in the period from January 1, 1990 to January 1, 2015.

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