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REVIEW

Genetic and epigenetic variations associated with idiopathic recurrent pregnancy loss

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Abstract

Recurrent pregnancy loss (RPL) is a reproductive disorder defined as two or more successive and spontaneous pregnancy losses (before 20 weeks of gestation), which affects approximately 1–2% of couples. At present, the causes of RPL remain unknown in a considerable number of cases, leading to complications in treatment and high levels of stress in couples. Idiopathic recurrent pregnancy loss (iRPL) has become one of the more complicated reproductive problems worldwide due to the lack of information about its etiology, which limits the counseling and treatment of patients. For that reason, iRPL requires further study of novel factors to provide scientific information for determining clinical prevention and targeted strategies. The aim of this study is to describe the most recent and promising progress in the identification of potential genetic and epigenetic risk factors for iRPL, expanding the genetic etiology of the disease.

Keywords Epigenetic · Genetic · Idiopathic recurrent pregnancy loss · Immune tolerance · Thrombophilia · Tissue remodeling

Introduction

Recurrent pregnancy loss (RPL) is a highly heterogeneous condition defined as two or more successive clinical pregnancy losses before 20 weeks of gestation and affects 1–2% of fertile women worldwide [1]. The etiology of the disease comprises different factors, such as chromosome abnormalities in the parents (2–5%), uterine alterations (10–15%), infections (0.5–5%), endocrinological disorders (17–20%), and autoimmune diseases (20%); nevertheless, approximately 50% of RPL cases remain unexplained (idiopathic) [1, 2].

Idiopathic recurrent pregnancy loss (iRPL) is a challenging condition that frustrates and adds emotional morbidity to couples and physicians due to a therapeutic dilemma that implies a lack of knowledge about the reason for repeated miscarriage and its correct management. Although some women suffering from this condition have good prognoses, in many other (often younger) patients, it underlies unknown diseases or poorly understood clinical conditions that give rise to poorer prognoses [3].

Research in the field of reproductive medicine is required and is important for providing iRPL patients with answers about their condition and better opportunities for targeted therapies for this disease. In this paper, we aim to summarize updated knowledge regarding the effects of genetic and epigenetic variants related to iRPL. Additionally, the goal of this paper is to specify and enumerate different causes linked to recurrent miscarriage and to reduce the uncertainty of the cases classified as idiopathic.

Methods

The literature was obtained by a global search of articles in the databases PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), Scopus (http://www.scopus.com/), and ScienceDirect (http://www.sciencedirect.com/). Idiopathic recurrent pregnancy loss, idiopathic recurrent miscarriage, unknown recurrent pregnancy loss, and unknown recurrent miscarriage keywords were used.

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We used the following as inclusion criteria: (1) original articles, reviews, or meta-analysis published from 2009 to 2016, (2) articles that indicate a possible genetic and epigenetic association with iRPL, (3) define RPL as two or more miscarriages, (4) any ethnic population, (5) articles were in English.

Parental chromosomal abnormalities Schuster and Ford established that parental cytogenetic abnormalities explain approximately 2–5% of RPL, with balanced Robertsonian and reciprocal chromosomal translocations being the most common alterations [1]. A comparable percentage has been validated by recent studies. For example, Sheth et al. reported that chromosomal abnormalities occur in 3.7% of RPL cases, with reciprocal translocations being the most common chromosomal abnormalities (24.7%), followed by Robertsonian translocations (17.64%), mosaicism (4.11%), small supernumerary marker chromosomes (4.7%), and interstitial microdeletions (0.6%) [4]. Ocak et al. found chromosomal abnormalities in 5.7% of parents with RPL, 92.9% of which were structural types [5]. Asgari et al. showed that 3.07% of couples with two miscarriages and 5.3% of couples with three miscarriages exhibit genetic abnormalities [6]. El-Dahtory reported a frequency of abnormalities in 6.1% of Egyptian couples with iRPL, with structural abnormalities being the most frequent alterations [7] (Fig. 1).

Chromosomal abnormalities in spontaneous abortion products The presence of chromosomal abnormalities is a common feature of cells from spontaneous abortion samples, and unlike those reported in parents, these tend to be numerical abnormalities and had a higher prevalence. This was shown by Ocak et al., who observed 31.9% of abnormalities (mostly numerical) in the tissue of analyzed abortions, compared to just 5.7% of abnormalities (mostly structural) in couples with RPL [5]. Furthermore, Marquard et al. reported aneuploidies (mainly trisomy) in 78% of spontaneous abortion products from women over 35 years of age who were affected by iRPL but did not detect any chromosomal abnormalities in the parents [8].

This confirms that aneuploidies in the abortion products may be involved with a large number of iRPL cases and that embryo karyotyping from patients with iRPL can provide significant benefits to patients. This has been shown by Hodes et al., who analyzed 2282 spontaneous abortion products from mothers with normal karyotypes and who were diagnosed with iRPL and found aneuploidies in 60% of these cases [9]. On the basis of these results, they proposed the use of preimplantation genetic screening as a useful preventive strategy that significantly reduced the miscarriage rate to just 6.9% by selecting euploid embryos for implantation [9].

The high incidence of aneuploidies in abortion material from iRPL cases shows strong evidence that this condition is mostly caused by numerical chromosome abnormalities in the fetus (Fig. 1) but also raises questions regarding the predisposing factors in couples suffering from iRPL. One explanation for this phenomenon may be the meiotic errors during spermatogenesis or oocyte maturation, because a high rate of errors has been associated with RPL and the occurrence of aneuploidies [10]. In this respect, some authors have indicated an involvement of synaptonemal complex protein 3 (SYCP3) alterations, as these are associated with reproductive problems and because this protein plays a critical role in synapse processes, recombination, and segregation of meiotic chromosomes [11, 12]. In this regard, Bolor et al. described mutations (denominated c.IVS7–16_19delACTT and c.657T → C) in SYCP3 gene that are associated with iRPL cases, and which this study proposes as risk factors for chromosomal nondisjunction and aneuploid embryo formation [11]. The authors suggested that these genetic variants can lead to an aberrant synaptonemal complex, abnormal chromosomal segregation, and chromosomal abnormalities [11]. Later, Sazegari et al. (2014) found that the T657C polymorphism in the SYCP3 gene may be a genetic factor for women with iRPL. Variants in the SYCP3 gene could alter the pairing and recombination of homologous chromosomes during meiosis and may lead to a high risk of embryo aneuploidy [12]. Nevertheless, as these variants are only present in a subset of women with iRPL, more studies are required to clarify the role of other variations in meiotic genes that could lead to chromosomal abnormalities in the gametes and embryos. Alternately, mutations in the SYCP3 gene in men are more commonly associated with azoospermia by meiotic arrest than with RPL [13]; nevertheless, Stouffs et al. reported a variant (c.548T > C) in the SYCP3 gene in one man from a couple suffering from recurrent miscarriage [14]. However, as this genetic change was present in only one case, further studies are required.

Telomere length in couples with iRLP Telomeres are the end parts of chromosomes, composed mainly of highly repetitive and noncoding DNA with an important role in the maintenance of chromosome integrity, cell division, and cell survival [15]. Due to this critical role in cellular homeostasis, the telomere length influences different disorders such as cancer, aging, and degenerative diseases [15]. Although its role in the risk of recurrent miscarriage has not been well-studied, Thilagavathi et al. reported that shorter telomere length in both members of a couple is associated with iRPL, as this can reduce the potential for cell replication in the blastocyst or embryo [16]. Similarly, Hanna et al. found that couples with RPL had shorter telomeres than controls and proposed that this could be related to a faster aging rate [17] (Fig. 1). This research highlights the telomere length as a potential factor in the disease; however, more studies are needed to confirm these results and determine the cause of telomere shortening in couples with iRPL.

Male factor The common etiology of RPL underestimates the effect of genetic sperm parameters in the disease, but some parameters such as damaged DNA integrity (DNA fragmentation), loss of organization, loss of chromatin compaction (nuclear chromatin decondensation), and chromosomal aneuploidy are associated with iRPL [18]. DNA fragmentation is the critical male factor associated with the disease and can contribute to unsuccessful pregnancy in couples, even in men with a normal quantity, motility, and morphology of spermatozoa [19]. The degree of DNA fragmentation is related to both infertility and iRPL; Kumar et al. found that men with iRPL show a higher DNA fragmentation index than fertile controls and proposed that a high percentage of sperm DNA fragmentation is associated with male infertility, whereas a lower percentage did not affect fertilization but increased the risk of pregnancy loss [20] affecting embryogenesis and causing problems in pregnancy maintenance [20, 21].

This DNA damage is primarily caused by oxidative stress, which is due to an increase in free radicals that have a negative impact on cell DNA integrity. Because of the effect of DNA fragmentation and the role of oxidative stress in iRPL, management by antioxidant supplement therapy in these patients would appear to be an important strategy for minimizing the risk of genetic damage to their gametes [21].

Alternately, recent studies have found that couples with iRPL have higher rates of sperm aneuploidy [18, 22], even though the male exhibits normal sperm parameters such as cell count and mobility [22]. Surprisingly, chromosomal polymorphisms such as variants of satellite size in the male acrocentric chromosomes may increase the risk of sperm aneuploidies and subsequent embryo loss [23].

Another male factor that may be associated with iRPL is the presence of microdeletions in the "azoospermia factor" regions (AZFa, AZFb, and AZFc) on the Y chromosome. The AZF regions on the Y chromosome have been related to defective spermatogenesis, giving rise to infertility problems. Associations between Y chromosome microdeletions and iRPL have been found in a study by Agarwal et al., where microdeletions were shown to be a possible cause of the disease [24]. Likewise, the study by Soleimanian et al. found associations between microdeletion of the AZFc region and RPL in Iranian men [25] (Fig. 1). However, it is necessary to investigate other male factors associated with iRPL, since these microdeletions have not always been observed in this type of patient [26] [27].

Effect of genetic disturbance

iRPL is a highly heterogenic disease. There are many genetic aberrations that can influence the development and progress of iRPL altering important biological pathways. Krieg et al. used a microarray technique validated by qRT-PCR to compare gene expression of deciduas from iRPL cases with controls (one normal term delivery sample and an aneuploid embryonic demise sample). Krieg et al. demonstrated deregulation of 155 genes involved in multiple processes such as the cell cycle, apoptosis, cell signaling, mobility, and immune response [28]. Similarly, Othmanet et al. identified 346 genes differentially expressed in the secretory endometrial lining from RPL cases and fertile women that are involved in angiogenesis, cell adhesion, cell cycle, cell differentiation, and embryonic morphogenesis [29]. These studies highlight the role of genetic disorders in RPL and indicate the importance of research in this field. Thus, in this paper, we want to show that particular variants of key genes can be related to recurrent miscarriages that have unknown etiologies.

Genetic variants can alter correct tissue formation and remodeling during pregnancy

For a successful pregnancy, structural and physiological changes in maternal tissues are required during embryo implantation, placentation, and pregnancy maintenance. For this reason, alterations due to point mutations and genetic polymorphisms in genes involved in embryo implantation and placentation could be risk factors for iRPL (Table 1).

Studies have found that polymorphisms in vascular endothelial growth factor (*VEGF*)-related genes might be associated with iRPL. The *VEFG* gene plays a key role in vascularization and angiogenesis (blood vessel formation), and it has been found to be downregulated in iRPL with possible effects on vascular dysfunction [74]. The rs35569394 (− 2549 I/D) and rs1570360 (-1154G > A) polymorphisms in the functional promoter region of the *VEGFA* gene have been proposed to be potential risk factors for iRPL [30, 31], although more studies are necessary to confirm this. Similarly, a case-control study found that the rs1870377 (1719A/T) polymorphism in the vascular endothelial growth factor receptor 2 (*VEGFR-2 or KDR*) is significantly associated with iRPL and may be a factor in disease susceptibility [32]. Cao et al. found that the rs6053283 polymorphism Prokineticin receptor 2 (*PKR2*), a receptor for the endocrine gland-derived vascular endothelial growth factor 2, was significantly associated with iRPL in the Chinese Han population probably due to its role in angiogenesis during pregnancy [33]. Additionally, meta-analysis studies have shown that rs1042522 (p53 Arg72Pro or p53 codon 72) polymorphisms in the *TP53* gene (another gene related to angiogenesis and embryo development) are related to the occurrence of iRPL [31, 34]. These studies support the hypothesis that vasculogenesis dysfunction during embryogenesis, which is promoted by gene alteration, may affect pregnancy and predisposition to iRPL.

Endothelial nitric oxide synthase (eNOS) is an enzyme that plays a role in vascular relaxation-contraction, which is

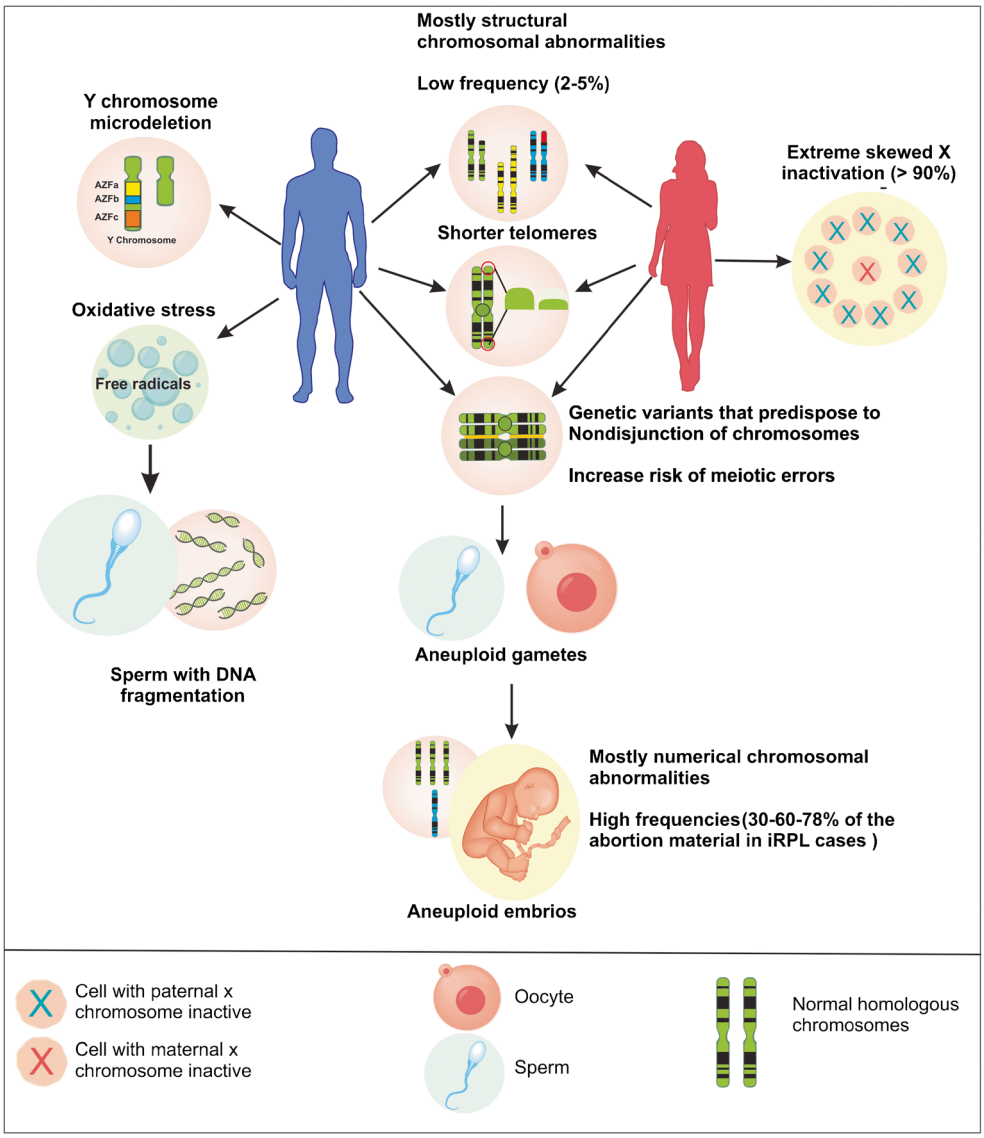

Fig. 1 Chromosomal abnormalities in recurrent pregnancy loss. Chromosomal abnormalities have been found to be associated with recurrent pregnancy loss at the level of the parents, gametes, and fetus. Numerical and structural abnormalities show the strongest evidence of a relationship with the disease

important to the implantation process because it facilitates correct perfusion and endometrial receptivity [74]. Recent studies have shown an association between downregulation of the eNOS enzyme and iRPL. Furthermore, the rs1799983 (G894T) polymorphism in the *eNOS* gene has been associated with iRPL [31, 35], although this association may vary between study populations [35].

Other enzymes of interest in iRPL are the matrix metalloproteinases (MMPs). MMPs are related to the degradation and remodeling of the endometrial extracellular matrix, which is an important event in decidualization, implantation, and placentation. Furthermore, it has been found that function al polymorphisms such as rs2285053 (− 735 C/T) in *MMP2* and rs34016235 (− 1562 C/T) in *MMP9* are associated with iRPL in women [36].

Similarly, it has been proposed that the rs1042838 (PROGIN or G/T - Val660Leu) polymorphism in the progesterone receptor (*PGR*) gene could be a factor that confers susceptibility to iRPL, since progesterone is important in different reproductive pathways such as oocyte maturation, implantation, and maintenance of the placenta [37].

Genetic variants that affect hemostasis and thrombophilia susceptibility

Different factors associated with susceptibility to iRPL are linked to genetically inherited thrombophilia, because the predisposition to improperly form blood clots can affect blood flow to the fetus and cause vasculopathy (Table 1). One of the most frequently studied genetic alterations that can lead to these disorders is in the gene for the methylenetetrahydrofolate reductase (*MTHFR*) that acts on the methionine cycle and folate metabolism affecting its enzymatic activity and leading

Gene	Common names	rs code	Biological effect	Reference
VEGFA	− 2549 I/D	rs35569394	Vascular function	[30]
VEGFA	-1154G > A	rs1570360	Vascular function	[31]
VEGFR-2	1719A/T	rs1870377	Vascular function	[32]
PKR2	Unspecified	rs6053283	Vascular function	[33]
TP53	p53 Arg72Pro or p53 codon 72	rs1042522	Vascular function and embryo development	[31, 34]
eNOS	G894T	rs1799983	Vascular relaxation-contraction	[31, 35]
MMP2	− 735 C/T	rs2285053	Remodeling of extracellular matrix endometrium	[36]
MMP9	− 1562 C/T	rs34016235	Remodeling of extracellular matrix endometrium	[36]
PGR	G/T - Val660Leu (PROGIN)	rs1042838	Oocyte maturation, implantation, and maintenance of the placenta	[37]
MTHFR	A1298C	rs1801131	Thrombophilia	[38–40]
MTHFR	C677T	rs1801133	Thrombophilia	[38–43]
F5	factor V Leiden	rs6025	Thrombophilia	[44, 45]
F2	G20210A	rs1799963	Thrombophilia	[46]
THBD	C1418T	rs1042579	Thrombophilia	[47]
EPCR	1652C /G	rs867186	Thrombophilia	[48]
SERPINC1	786G > A	rs2227589	Thrombophilia	[49]
F13A1	Val34Leu-G103T	rs5985	Thrombophilia	[50]
F13A1	Y205F-A614T	rs3024477	Thrombophilia	[51]
F13A1	C1694T or Pro564Leu	rs5982	Thrombophilia	[51]
PAI-1	4G/5G	rs1799889	Thrombophilia	[45, 50, 52–55]
PAI-1	-844G > A	rs2227631	Thrombophilia	[53]
PAI-1	11053T > G	rs7242	Thrombophilia	[53]
ACE	I/D	rs1799752	Thrombophilia	[56, 57]
IL-1β	− 511T > C	rs16944	Immune tolerance	[58]
IL-17	G-197A	rs2275913	Immune tolerance	[59]
IL-18	137G/C	rs187238	Immune tolerance	[60]
IL-10	− 819 C/T	rs1800871	Immune tolerance	[61]
IL-10	2195 A > G	rs1518111	Immune tolerance	[62]
IL-6	-634C/G	rs1800796	Immune tolerance	[63, 66]
TGF-β1	G915C or Arg25Pro	rs1800471	Immune tolerance	[64]
TNF-α	− 863C > A	rs1800630	Immune tolerance	[65]
CTLA-4	+ 49A/G	rs232775	Immune tolerance	[66]
FOXP3	− 924 A/G	rs2232365	Immune tolerance	[67]
FOXP4	− 3279 C/A	rs3761548	Immune tolerance	[67]
FOXP5	del/ATT	rs5902434	Immune tolerance	[67]
FOXP6	Unspecified	rs2294021	Immune tolerance	[67]
SELP	C-2123G or N562D	rs6127	Immune tolerance	[68]
DICER	Unspecified	rs3742330	Epigenetic	[69]
DROSHA	Unspecified	rs10719	Epigenetic	[69]
XPO5	Unspecified	rs11077	Epigenetic	[69]
RAN	Unspecified	rs14035	Epigenetic	[69]
MIR125a	Unspecified	rs12976445	Epigenetic	[70, 71]
MIR125a	Unspecified	rs41275794	Epigenetic	[70, 71]
MIR423	Unspecified	rs6505162	Epigenetic	[72]
MIR27a	Unspecified	rs895819	Epigenetic	[73]
MIR449b	Unspecified	rs10061133	Epigenetic	[73]

to the clinical condition hyperhomocysteinemia. Although the association of polymorphisms in this gene with the disease is still controversial, some case-control studies have found an association in the *MTHFR* rs1801131 (A1298C) and rs1801133 (C677T) polymorphisms with iRPL [38, 39]. Additionally, a meta-analysis by Yang et al. concluded that both polymorphisms are associated with hyperhomocysteinemia [40], while the meta-analysis by Chen et al., Yunlei et al., and Wu et al. identified an association with the C677T polymorphism but not with the A1298C polymorphism [41–43]. Nevertheless, it is important to consider that this association may vary depending on the study population [41, 42].

On the other hand, genetic variations in key molecules of the coagulation cascade can be involved in predisposition to iRPL (Fig. 2). Genetic variations in coagulation factor V (FV) have been suggested to be a risk for iRPL. In the coagulation cascade, the active form of FV causes coagulant effects by catalyzing the conversion of prothrombin to thrombin, which is normally inhibited by active protein C (APC). The best-studied mutation in the FV gene (*F5*) is the rs6025 variant, also known as factor V Leiden (FVL), which leads to a predisposition to the formation of blood clots, because it confers resistance to inactivation by APC [44, 45]. Recent studies have found that it may be a risk factor for iRPL in both parents, because the father can transmit the FVL allele (which is autosomal dominant) to the fetus, causing thrombosis in fetal blood vessels [44, 45]. Nevertheless, not all studies have found associations between FVL and iRPL; thus, a recent study involving Brazilian women did not detect significant differences in FVL allele frequencies between cases and controls [75].

Additionally, there is a possible association between the rs1799963 (G20210A) polymorphism in the prothrombin (a precursor of thrombin) gene (*F2*) and iRPL in women, as these mutations raise the level of thrombogenic precursors leading to an increased risk of placental thrombophilia [46]. Nevertheless, Gonçalves et al. found no significant differences in the genetic frequency of these variants between cases with iRPL and controls in a sample of Brazilian women [75].

Variants of inhibitors of the coagulation process may have an association with iRPL. APC activator polymorphisms, such as thrombomodulin gene (*THBD*) rs1042579 (C1418T) and endothelial protein C receptor gene (*EPCR*) rs867186 (1652C/G) have been proposed to be risk factors for iRPL due to the association of these variants with lower levels of soluble proteins impeding the adequate regulation of coagulation processes [47, 48]. Similarly, the rs2227589 (786G > A) polymorphism in the antithrombin (*AT or SERPINC1*) gene has been associated with an increased risk of iRPL, as these proteins inhibit the transition of prothrombin to thrombin and avoid the coagulation process [49].

Finally, the rs5985 (G103T or Val34Leu), rs3024477 (A614T or Y205F) and rs5982 (C1694T or Pro564Leu) polymorphisms in coagulation Factor XIII (*F13*) have been associated with iRPL, as this factor is important in the final steps of the coagulation cascade because of its role in cross-linking fibrin and giving stability to the clot [50, 51]. The variant Val34Leu of FXIII appears to increase enzyme activity and the stability of the clot, which would lead to an increase in thrombophilia risk, but the A614T and C1694T variants appear to be associated with decreased levels and reduced enzyme activity, which may contradict this hypothesis; however, FXIII is not only important in coagulation processes but also in tissue remodeling during pregnancy [50, 51].

On the other hand, studies have shown the importance of genes that control the fibrinolysis process (degradation of fibrin by plasmin enzyme, leading to the dilution of thrombus), such as the plasminogen activator inhibitor-1 (*PAI-1*) gene that impedes fibrinolysis through inhibition of the enzymes involved in the conversion of plasminogen to plasmin. Several studies have reported an association between the *PAI-1* rs1799889 (4G/5G) polymorphism and iRPL, because this polymorphism can generate high levels of its protein and increase resistance to fibrinolysis [45, 50, 52–54, 55]. Salazar et al. showed that this genetic variant can alter the metabolic and immunological profiles of patients with iRPL [54]. Although a meta-analysis of this polymorphism conducted by Su et al. failed to find an association with iRPL, the authors suggested that it was due to the high heterogeneity of the studies and emphasized the importance of *PAI-1* in the fibrinolysis process, not only in the removal of thrombus but also in the processes of tissue remodeling during embryo implantation and placentation [57]. Similarly, a case-control study of a Polish population conducted by Kurzawińska et al. did not find a significant difference in the polymorphism frequency between cases of unknown RPL vs controls [82]. Finally, other polymorphisms such as rs2227631 (-844G > A) and rs7242 (11053T > G) in *PAI-1* have been proposed as risk factors for the disease in Korean women [53].

Associations with iRPL have also been found for the rs1799752 (I/D) (insertion or deletion in intron 19 of 287-bps) polymorphism in the angiotensin-converting enzyme (*ACE*) gene due to its importance in vasoconstriction and regulation of *PAI-1* activity, which has been associated with iRPL in a meta-analysis conducted by Su et al. [57]. Likewise, Fazelnia et al. observed an association between the ACE (I/D) variant with iRPL in Iranian women [56]. However, other studies in Iranian and Polish populations did not find an association between these polymorphisms and iRPL [55, 82], indicating that more research is required to establish this association.

Genetic variants of immune tolerance

Fetal tissues and cells are semi-allogeneic in the uterus and certain processes should be addressed to correct immune

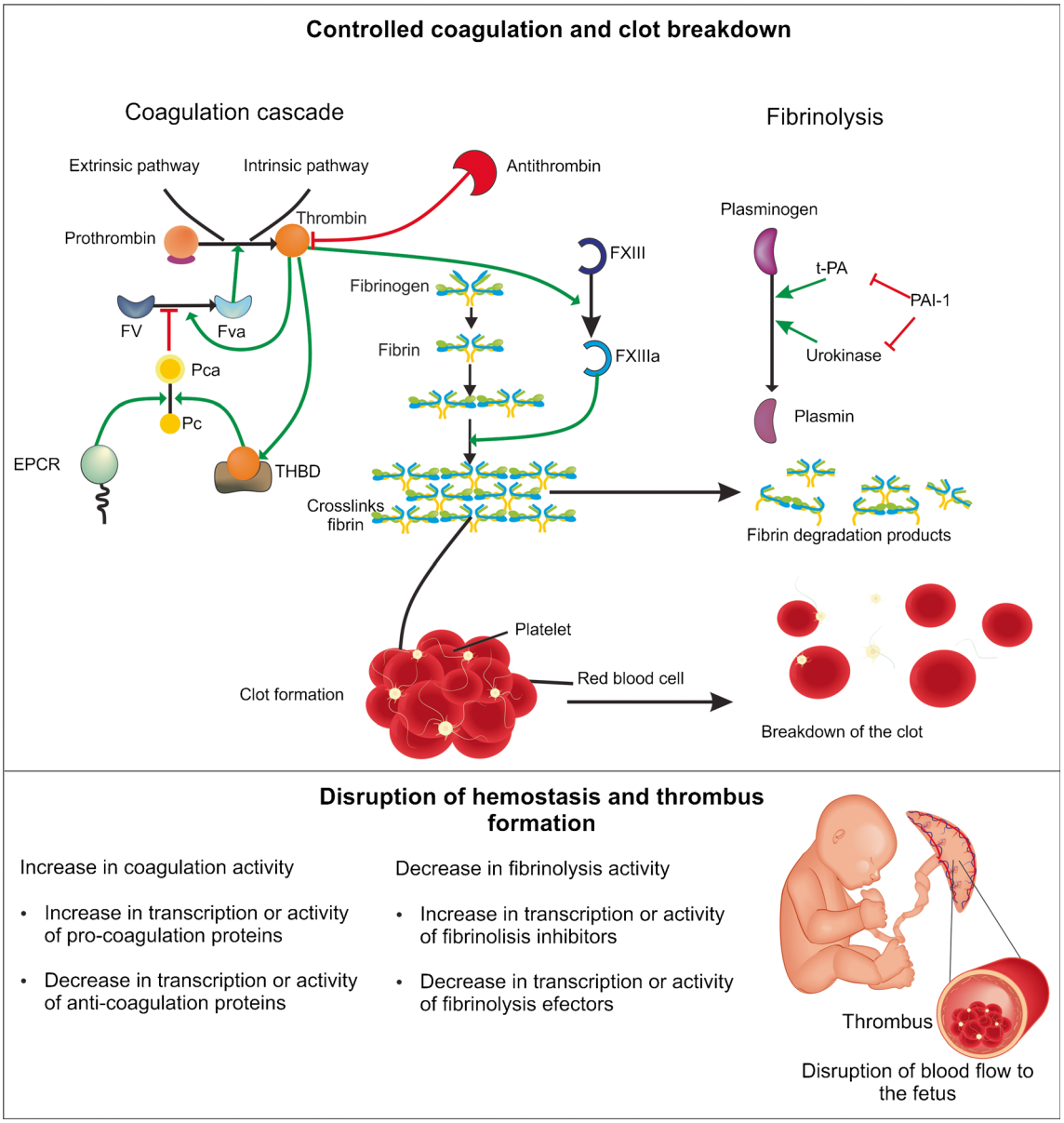

Fig. 2 Role of hemostasis dysregulation in iRPL. Schematic representation of the coagulation and fibrinolysis processes and the role of key proteins affected in iRPL. Disruption of the hemostasis process in iRPL by increases in procoagulant activity and loss of anticoagulant controllers leads to the formation of thrombus in the mother-fetus vessels

tolerance to the fetus. It has been proposed that the activation of inflammatory processes of immune rejection and the suppression of immune regulators contribute to iRPL [76, 77]. Studies have demonstrated genetic variations in interleukin (IL) genes responsible for stimulating and regulating the activity of the immune system (Table 1). The importance of pro-inflammatory IL genes such as the *IL-1β* rs16944 (− 511T > C) polymorphism has been associated with iRPL in Korean women, and it has been involved with an increased activity of NK (natural killer) cells leading to a greater immune response [58]. Likewise, the rs2275913 (G-197A) polymorphism in the *IL-17* gene has been associated with an increased miscarriage risk [59], and higher levels of IL-17 have been found in women with iRPL [77, 59]. Similarly, Chen et al. reported that the rs187238 (137G/C) polymorphism in the *IL-18* gene has also been related to an increased risk of iRPL, because this variant is associated with high transcriptional activity of the IL-18 gene that stimulates the immune response [60].

Likewise, variants in anti-inflammatory ILs such as the rs1518111 (2195 A > G) and rs1800871 (− 819 C/T) polymorphisms in the *IL-10* gene have been shown to lead to an increased risk of iRPL, as IL-10 regulates the immune response and its secretion has been considered essential for successful pregnancy [61, 62]. In addition, research with Iranian women has shown a relationship between the rs1800796 (-634C/G) polymorphism in the *IL-6* gene and an increased risk of iRPL,

because IL-6 plays a role in reproduction and immune balance, acting as a multifunctional cytokine with pro and anti-inflammatory properties and mediates the balance between T helper (Th)-17 and Treg cells [63, 66].

Different genetic variants in cytokines may affect iRPL susceptibility, such as the polymorphism rs1800471 (G915C or Arg25Pro) in the transforming growth factor beta 1 (*TGF-β1*) gene that acts as an anti-inflammatory cytokine with a critical role in the control of T and B lymphocytes [64]. Alternately, the rs1800630 (− 863C > A) polymorphism in the tumor necrosis factor-alpha (*TNF-α*) gene, a pro-inflammatory cytokine, was associated with an increased risk of iRPL in Korean women [65].

Additional genetic variants such as the human leukocyte antigen E (HLA-E * 0101) polymorphism can increase susceptibility to the disease, since it is one of the few HLAs expressed in the fetal trophoblast and has a critical role in the regulation of NK cells [83]. Additionally, the rs232775 (+49A/G) polymorphism in the cytotoxic T-lymphocyte antigen 4 (*CTLA-4*) gene may be a risk factor for iRPL, as this molecule is an important antigen for regulation of the T cell response and for immune tolerance [66].

Recent studies have demonstrated a possible association between iRPL and the rs2232365 (− 924 A/G), rs3761548 (− 3279 C/A), rs5902434 (del/ATT) and rs2294021 variants of the transcription factor Forkhead Box P3 (*FOXP3*) gene, an essential protein for the development of regulatory T cells and suppression of the immune response [67]. Similarly, the rs6127 (C-2123G or N562D) polymorphism in P-selectin (*SELP*) may play a role in susceptibility to iRLP, since this protein is important in leukocyte recruitment [68]. Recent evidence shows the importance of immune response regulation for successful pregnancy and the relationships between genetic variants and increased risk of iRPL that are related to the correct balance between pro-inflammatory and immune regulatory molecules.

Epigenetic deregulation in iRPL

Epigenetic markers are responsible for controlling correct gene expression to ensure cellular and tissue homeostasis. Modifications in epigenetic machinery play an important role in the biological regulation of many diseases, such as iRPL, as crucial changes in epigenetic markers during pregnancy are necessary for embryo implantation, tissue remodeling, and pregnancy maintenance. Although there is limited information about the role of epigenetic alterations in multiple miscarriages, these changes have been implicated in reproductive complications [78].

Among the different alterations, the skewed X chromosome inactivation (SXCI) has been associated with iRPL. The inactivation of one X chromosome is a phenomenon that occurs in female mammals as a mechanism of dosage compensation that consists of random inactivation of one of the two X chromosomes (maternal or paternal) [79]. With X chromosome inactivation, 50% of the somatic cells in an individual carry the inactivated paternal X chromosome and 50% carry an inactivated maternal X chromosome. When this process does not occur randomly, females have one chromosome (maternal or paternal), which is more frequently inactivated than the other. When 75–80% of cells are inactivated, the same X chromosome is defined as SXCI and when this occurs in 90%–95% of cells, it is defined as extreme SXCI (ESXCI) [80, 81].

There is no clear consensus about the association of SXCI and iRPL, but a meta-analysis conducted by Sui et al. showed that there is an association between ESXCI and iRPL for women with three or more miscarriages [84]. However, no significant association was found when the degree of SXCI was lower (< 90%) or when RPL was defined as two or more miscarriages [84]. While Su et al. showed that ESXCI was significantly and consistently associated with iRPL, this association was found both when RPL was defined as either two or three miscarriages [37]. Given the recent reports, ESXCI may play a critical role in the unknown etiology of RLP, but further studies are needed on the molecular, cellular, and physiological implications of this abnormal inactivation.

Another epigenetic phenomenon of interest in understanding iRPL is the pattern of DNA methylation (addition of a methyl group to cytosine), which is a critical process for silencing specific genes in order to maintain cellular homeostasis and identity [85]. In this regard, Hanna et al. found differences in the methylation patterns in specific loci associated with recurrent abortion, imprinted genes, and immune signaling pathways in placenta samples from patients with iRPL [86].

Interest in research on microRNAs (miRNAs) has increased in recent years. miRNAs are molecules that interfere with the translation of specific target genes having an important role in gene expression regulation. Jung et al. found an association between iRPL and polymorphisms in coding genes for key proteins in miRNA biogenesis, such as rs3742330 in *DICER*, rs10719 in *DROSHA*, rs11077 in exportin-5 (*XPO5*), and rs14035 in RAN GTPase (*RAN*) [69]. Additionally, other studies have shown an association between the disease and variants in genes encoding miR-125a (rs12976445 and rs41275794), miR-423 (rs6505162), miR-27a (rs895819), and miR-449b (rs10061133). Because these polymorphisms can deregulate miRNAs levels or activity, leading to inadequate regulation of the miRNAs target genes, which are related to embryonic development and maintenance of pregnancy [70–73]. Similarly, higher levels of miR-16 have been found in villi and deciduas of iRPL patients, which could affect pregnancy as this miRNA target

VEGF inhibiting its expression and negatively affecting the placentation and angiogenesis processes [87].

Long noncoding RNAs (lncRNs) are other factors of interest in the epigenetic control of gene expression and research on these RNAs may contribute to understanding the mechanisms involved in iRPL. A recent study identified 1449 lncRNAs that are differentially expressed between women with RPL and healthy controls [88]. The authors also indicated that the deregulated lncRNAs are primarily involved in endocrine, immunity, cellular-extracellular matrix interaction and cell apoptosis pathways [88].

Discussion and conclusions

iRPL is a multifactorial disease with a large percentage of cases with unknown causes giving rise to difficulties in treatment and leading to more pressure and stress in infertile couples. This has led to research to determine the etiology of recurrent pregnancy seeking to improve treatments and preventive strategies (Fig. 3).

The only consensus of genetic factors considered to be associated with RLP that explain 2–5% of the cases are chromosomal abnormalities (mostly structural) in the parents. In addition, aneuploidy of the developing embryo is quite common in RPL, especially with idiopathic losses, showing strong evidence of its relationship with the disease and considered to be the major cause of iRPL.

Additionally, different chromosomal alterations not detected by traditional cytogenetic techniques such as the length of telomeres, skewed X inactivation, sperm DNA fragmentation, and microdeletions in the Y chromosome have been found to be associated with risk of iRPL. However, published literature is still controversial with regard to its association with iRPL due to differences in results from various studies, populations, and definitions of the pathology (defined as two or three miscarriages).

A number of studies have investigated specific gene variants that can predispose couples to iRPL that affect essential processes in pregnancy such as implantation, placentation, blood vessel formation, maintenance of hemostasis, and immune tolerance. However, the results are inconclusive as a consequence of uncommon genetic variants in particular populations. Therefore, we conclude that iRPL is a highly multifactorial disease that apparently includes the deregulation of key genes involved in pregnancy. Nevertheless, due to its multifactorial etiology, complex characteristics and differences in prevalence of genetic variants between populations, it is difficult to establish with confidence the risk factors or biological or molecular markers.

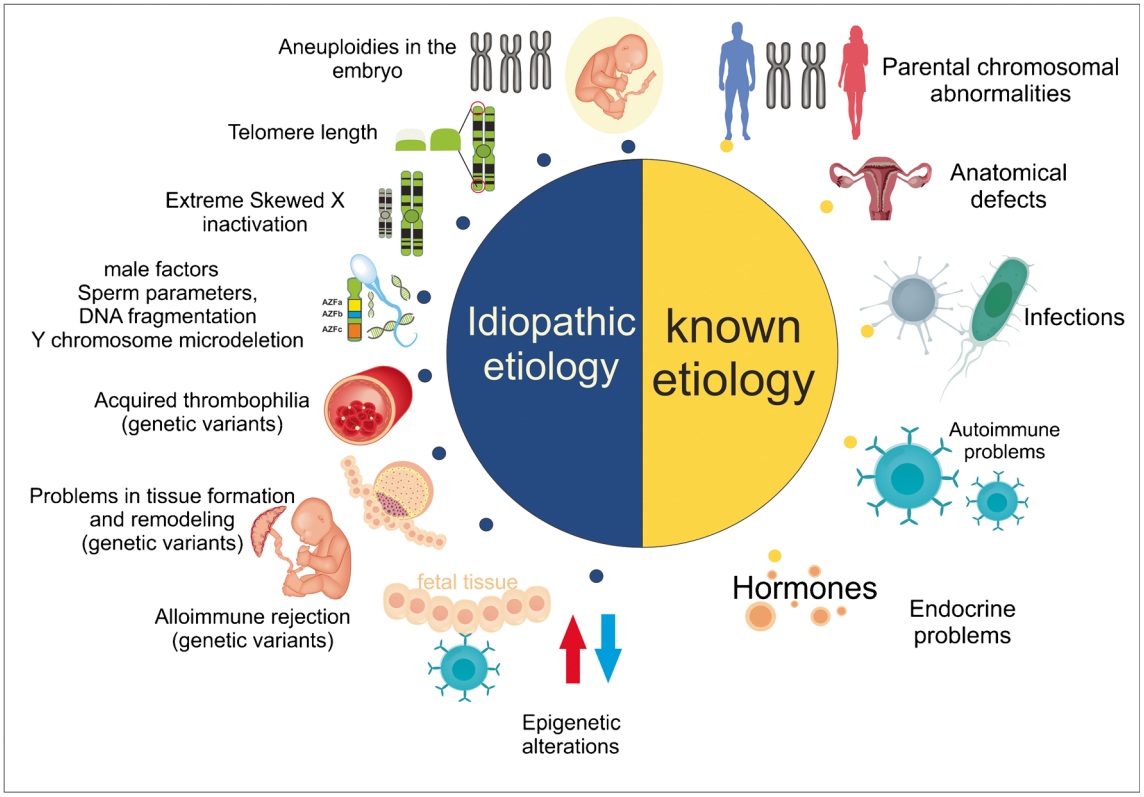

Fig. 3 Associated factors of RPL. The known etiology includes proven causes of RPL. The idiopathic etiology is limited to cases in which there is no scientific consensus, but recent studies have found associations with the disease

Compliance with ethical standards

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

References

1. Ford HB, Schust DJ. Recurrent pregnancy loss: etiology, diagnosis, and therapy. Rev Obstet Gynecol. 2009;2:76–83.
2. Jeve YB, Davies W. Evidence-based management of recurrent miscarriages. J Hum Reprod Sci. 2014;7:159–69. https://doi.org/10.4103/0974-1208.142475.
3. Saravelos SH, Li T-C. Unexplained recurrent miscarriage: how can we explain it? Hum Reprod. 2012;27:1882–6. https://doi.org/10.1093/humrep/des102.
4. Sheth FJ, Liehr T, Kumari P, Akinde R, Sheth HJ, Sheth JJ. Chromosomal abnormalities in couples with repeated fetal loss: an Indian retrospective study. Indian J Hum Genet. 2013;19:415–22. https://doi.org/10.4103/0971-6866.124369.
5. Ocak Z, Özlü T, Ozyurt O. Association of recurrent pregnancy loss with chromosomal abnormalities and hereditary thrombophilias. Afr Health Sci. 2013;13:447–52. https://doi.org/10.4314/ahs.v13i2.35.
6. Asgari A, Ghahremani S, Saeedi S, Kamrani E. The study of chromosomal abnormalities and heteromorphism in couples with 2 or 3 recurrent abortions in Shahid Beheshti Hospital of Hamedan. Iran J Reprod Med. 2013;11:201–8.
7. El-Dahtory FAM. Chromosomal abnormalities as a cause of recurrent abortions in Egypt. Indian J Hum Genet. 2011;17:82–4. https://doi.org/10.4103/0971-6866.86186.
8. Marquard K, Westphal LM, Milki AA, Lathi RB. Etiology of recurrent pregnancy loss in women over the age of 35 years. Fertil Steril. 2010;94:1473–7. https://doi.org/10.1016/j.fertnstert.2009.06.041.
9. Hodes-Wertz B, Grifo J, Ghadir S, Kaplan B, Laskin CA, Glassner M, et al. Idiopathic recurrent miscarriage is caused mostly by aneuploid embryos. Fertil Steril. 2012;98:675–80. https://doi.org/10.1016/j.fertnstert.2012.05.025.
10. McCoy RC, Demko ZP, Ryan A, Banjevic M, Hill M, Sigurjonsson S, et al. Evidence of selection against complex mitotic-origin aneuploidy during preimplantation development. PLoS Genet. 2015;11:e1005601. https://doi.org/10.1371/journal.pgen.1005601.
11. Bolor H, Mori T, Nishiyama S, Ito Y, Hosoba E, Inagaki H, et al. Mutations of the SYCP3 gene in women with recurrent pregnancy loss. Am J Hum Genet. 2009;84:14–20. https://doi.org/10.1016/j.ajhg.2008.12.002.
12. Sazegari A, Kalantar SM, Pashaiefar H, Mohtaram S, Honarvar N, Feizollahi Z, et al. The T657C polymorphism on the SYCP3 gene is associated with recurrent pregnancy loss. J Assist Reprod Genet. 2014;31:1377–81. https://doi.org/10.1007/s10815-014-0272-6.
13. Miyamoto T, Minase G, Shin T, Ueda H, Okada H, Sengoku K. Human male infertility and its genetic causes. Reprod Med Biol. 2017;16:81–8. https://doi.org/10.1002/rmb2.12017.
14. Stouffs K, Vandermaelen D, Tournaye H, Liebaers I, Lissens W. Mutation analysis of three genes in patients with maturation arrest of spermatogenesis and couples with recurrent miscarriages. Reprod BioMed Online. 2011;22:65–71. https://doi.org/10.1016/j.rbmo.2010.08.004.
15. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet. 2005;6:611–22. https://doi.org/10.1038/nrg1656.
16. Thilagavathi J, Mishra SS, Kumar M, Vemprala K, Deka D, Dhadwal V, et al. Analysis of telomere length in couples experiencing idiopathic recurrent pregnancy loss. J Assist Reprod Genet. 2013;30:793–8. https://doi.org/10.1007/s10815-013-9993-1.
17. Hanna CW, Bretherick KL, Gair JL, Fluker MR, Stephenson MD, Robinson WP. Telomere length and reproductive aging. Hum Reprod. 2009;24:1206–11. https://doi.org/10.1093/humrep/dep007.
18. Zidi-Jrah I, Hajlaoui A, Mougou-Zerelli S, Kammoun M, Meniaoui I, Sallem A, et al. Relationship between sperm aneuploidy, sperm DNA integrity, chromatin packaging, traditional semen parameters, and recurrent pregnancy loss. Fertil Steril. 2015;105:58–64. https://doi.org/10.1016/j.fertnstert.2015.09.041.
19. Bareh GM, Jacoby E, Binkley P, Chang T-CA, Schenken RS, Robinson RD. Sperm deoxyribonucleic acid fragmentation assessment in normozoospermic male partners of couples with unexplained recurrent pregnancy loss: a prospective study. Fertil Steril. 2016;105:329–36.e1. https://doi.org/10.1016/j.fertnstert.2015.10.033.
20. Kumar K, Deka D, Singh A, Mitra DK, Vanitha BR, Dada R. Predictive value of DNA integrity analysis in idiopathic recurrent pregnancy loss following spontaneous conception. J Assist Reprod Genet. 2012;29:861–7. https://doi.org/10.1007/s10815-012-9801-3.
21. Imam SN, Shamsi MB, Kumar K, Deka D, Dada R. Idiopathic recurrent pregnancy loss: role of paternal factors; a pilot study. J Reprod Infertil. 2011;12:267–76.
22. Ramasamy R, Scovell JM, Kovac JR, Cook PJ, Lamb DJ, Lipshultz LI. Fluorescence in situ hybridization detects increased sperm aneuploidy in men with recurrent pregnancy loss. Fertil Steril. 2015;103:906–9.e1. https://doi.org/10.1016/j.fertnstert.2015.01.029.
23. Morales R, Lledó B, Ortiz JA, Ten J, Llácer J, Bernabeu R. Chromosomal polymorphic variants increase aneuploidies in male gametes and embryos. Syst Biol Reprod Med. 2016;62:317–24. https://doi.org/10.1080/19396368.2016.1212949.
24. Agarwal S, Agarwal A, Khanna A, Singh K. Microdeletion of Y chromosome as a cause of recurrent pregnancy loss. J Hum Reprod Sci. 2015;8:159–64. https://doi.org/10.4103/0974-1208.165145.
25. Soleimanian S, Kalantar SM, Sheikhha MH, Zaimy MA, Rasti A, Fazli H. Association between Y-chromosome AZFc region microdeletions with recurrent miscarriage. Iran J Reprod Med. 2013;11:431–4.
26. Ghorbian S, Saliminejad K, Sadeghi MR, Javadi GR, Kamali K, Amirjannati N, et al. The association between Y chromosome microdeletion and recurrent pregnancy loss. Iran Red Crescent Med J. 2012;14:358–62.
27. Wettasinghe TK, Jayasekara RW, Dissanayake VHW. Y chromosome microdeletions are not associated with spontaneous recurrent pregnancy loss in a Sinhalese population in Sri Lanka. Hum Reprod. 2010;25:3152–6. https://doi.org/10.1093/humrep/deq271.
28. Krieg SA, Fan X, Hong Y, Sang Q-X, Giaccia A, Westphal LM, et al. Global alteration in gene expression profiles of deciduas from women with idiopathic recurrent pregnancy loss. Mol Hum Reprod. 2012;18:442–50. https://doi.org/10.1093/molehr/gas017.
29. Othman R, Omar MH, Shan LP, Shafiee MN, Jamal R, Mokhtar NM. Microarray profiling of secretory-phase endometrium from patients with recurrent miscarriage. Reprod Biol. 2012;12:183–99. https://doi.org/10.1016/S1642-431X(12)60085-0.
30. Pereza N, Ostojić S, Smirčić A, Hodžić A, Kapović M, Peterlin B. The -2549 insertion/deletion polymorphism in the promoter region of the VEGFA gene in couples with idiopathic recurrent spontaneous abortion. J Assist Reprod Genet. 2015;32 https://doi.org/10.1007/s10815-015-0593-0.
31. Su M-T, Lin S-H, Chen Y-C. Genetic association studies of angiogenesis- and vasoconstriction-related genes in women with recurrent pregnancy loss: a systematic review and meta-analysis.

Hum Reprod Update. 2011;17:803–12. https://doi.org/10.1093/humupd/dmr027.
32. Su M-T, Lin S-H, Chen Y-C, Kuo P-L. Gene-gene interactions and gene polymorphisms of VEGFA and EG-VEGF gene systems in recurrent pregnancy loss. J Assist Reprod Genet. 2014;31:699–705. https://doi.org/10.1007/s10815-014-0223-2.
33. Cao Y, Zhang Z, Wang J, Miao M, Xu J, Shen Y, et al. Association between polymorphisms of prokineticin receptor (PKR1 rs4627609 and PKR2 rs6053283) and recurrent pregnancy loss. J Zhejiang Univ Sci B. 2016;17:218–24. https://doi.org/10.1631/jzus.B1500180.
34. Chen H, Yang X, Wang Z. Association between p53 Arg72Pro polymorphism and recurrent pregnancy loss: an updated systematic review and meta-analysis. Reprod BioMed Online. 2015;31:149–53. https://doi.org/10.1016/j.rbmo.2015.05.003.
35. Cao Y, Zhang Z, Xu J, Wang J, Yuan W, Shen Y, et al. Genetic association studies of endothelial nitric oxide synthase gene polymorphisms in women with unexplained recurrent pregnancy loss: a systematic and meta-analysis. Mol Biol Rep. 2014;41:3981–9. https://doi.org/10.1007/s11033-014-3266-7.
36. Pereza N, Ostojić S, Volk M, Kapović M, Peterlin B. Matrix metalloproteinases 1, 2, 3 and 9 functional single-nucleotide polymorphisms in idiopathic recurrent spontaneous abortion. Reprod BioMed Online. 2012;24:567–75. https://doi.org/10.1016/j.rbmo.2012.01.008.
37. Su M-T, Lin S-H, Chen Y-C. Association of sex hormone receptor gene polymorphisms with recurrent pregnancy loss: a systematic review and meta-analysis. Fertil Steril. 2011;96:1435–44.e1. https://doi.org/10.1016/j.fertnstert.2011.09.030.
38. Cao Y, Zhang Z, Zheng Y, Yuan W, Wang J, Liang H, et al. The association of idiopathic recurrent early pregnancy loss with polymorphisms in folic acid metabolism-related genes. Genes Nutr. 2014;9:402. https://doi.org/10.1007/s12263-014-0402-x.
39. Nair RR, Khanna A, Singh K. MTHFR C677T polymorphism and recurrent early pregnancy loss risk in north Indian population. Reprod Sci. 2012;19:210–5. https://doi.org/10.1177/1933719111417888.
40. Yang Y, Luo Y, Yuan J, Tang Y, Xiong L, Xu M, et al. Association between maternal, fetal and paternal MTHFR gene C677T and A1298C polymorphisms and risk of recurrent pregnancy loss: a comprehensive evaluation. Arch Gynecol Obstet. 2015; https://doi.org/10.1007/s00404-015-3944-2.
41. Wu X, Zhao L, Zhu H, He D, Tang W, Luo Y. Association between the MTHFR C677T polymorphism and recurrent pregnancy loss: a meta-analysis. Genet Test Mol Biomarkers. 2012;16:806–11. https://doi.org/10.1089/gtmb.2011.0318.
42. Chen H, Yang X, Lu M. Methylenetetrahydrofolate reductase gene polymorphisms and recurrent pregnancy loss in China: a systematic review and meta-analysis. Arch Gynecol Obstet. 2015;293:283–90. https://doi.org/10.1007/s00404-015-3894-8.
43. Cao Y, Xu J, Zhang Z, Huang X, Zhang A, Wang J, et al. Association study between methylenetetrahydrofolate reductase polymorphisms and unexplained recurrent pregnancy loss: a meta-analysis. Gene. 2013;514:105–11. https://doi.org/10.1016/j.gene.2012.10.091.
44. Udry S, Aranda FM, Latino JO, de Larrañaga GF. Paternal factor V Leiden and recurrent pregnancy loss: a new concept behind fetal genetics? J Thromb Haemost. 2014;12:666–9.
45. Ozdemir O, Yenicesu GI, Silan F, Köksal B, Atik S, Ozen F, et al. Recurrent pregnancy loss and its relation to combined parental thrombophilic gene mutations. Genet Test Mol Biomarkers. 2012;16:279–86. https://doi.org/10.1089/gtmb.2011.0191.
46. Gao H, Tao F. Prothrombin G20210A mutation is associated with recurrent pregnancy loss: a systematic review and meta-analysis update. Thromb Res. 2015;135:339–46. https://doi.org/10.1016/j.thromres.2014.12.001.
47. Cao Y, Zhang Z, Xu J, Yuan W, Wang J, Huang X, et al. The association of idiopathic recurrent pregnancy loss with polymorphisms in hemostasis-related genes. Gene. 2013;530:248–52. https://doi.org/10.1016/j.gene.2013.07.080.
48. Dendana M, Messaoudi S, Hizem S, Jazia KB, Almawi WY, Gris J-C, et al. Endothelial protein C receptor 1651C/G polymorphism and soluble endothelial protein C receptor levels in women with idiopathic recurrent miscarriage. Blood Coagul Fibrinolysis. 2012;23:30–4. https://doi.org/10.1097/MBC.0b013e328349cae5.
49. Guerra-Shinohara EM, Bertinato JF, Tosin Bueno C, Cordeiro da Silva K, Burlacchini de Carvalho MH, Pulcineli Vieira Francisco R, et al. Polymorphisms in antithrombin and in tissue factor pathway inhibitor genes are associated with recurrent pregnancy loss. Thromb Haemost. 2012;108:693–700. https://doi.org/10.1160/TH12-03-0177.
50. Elmahgoub IR, Afify RA, Abdel Aal AA, El-Sherbiny WS. Prevalence of coagulation factor XIII and plasminogen activator inhibitor-1 gene polymorphisms among Egyptian women suffering from unexplained primary recurrent miscarriage. J Reprod Immunol. 2014;103:18–22. https://doi.org/10.1016/j.jri.2014.02.007.
51. Jeddi-Tehrani M, Torabi R, Mohammadzadeh A, Arefi S, Keramatipour M, Zeraati H, et al. Investigating association of three polymorphisms of coagulation factor XIII and recurrent pregnancy loss. Am J Reprod Immunol. 2010;64:212–7. https://doi.org/10.1111/j.1600-0897.2010.00838.x.
52. Patil R, Ghosh K, Vora S, Shetty S. Inherited and acquired thrombophilia in Indian women experiencing unexplained recurrent pregnancy loss. Blood Cells Mol Dis. 2015;55:200–5. https://doi.org/10.1016/j.bcmd.2015.06.008.
53. Jeon YJ, Kim YR, Lee BE, Choi YS, Kim JH, Shin JE, et al. Genetic association of five plasminogen activator inhibitor-1 (PAI-1) polymorphisms and idiopathic recurrent pregnancy loss in Korean women. Thromb Haemost. 2013;110:742–50. https://doi.org/10.1160/TH13-03-0242.
54. Salazar Garcia MD, Sung N, Mullenix TM, Dambaeva S, Beaman K, Gilman-Sachs A, et al. Plasminogen activator inhibitor-1 4G/5G polymorphism is associated with reproductive failure: metabolic, hormonal, and immune profiles. Am J Reprod Immunol. 2016;76:70–81. https://doi.org/10.1111/aji.12516.
55. Shakarami F, Akbari MT, Zare Karizi S. Association of plasminogen activator inhibitor-1 and angiotensin converting enzyme polymorphisms with recurrent pregnancy loss in Iranian women. Iran J Reprod Med. 2015;13:627–32.
56. Fazelnia S, Farazmandfar T, Hashemi-Soteh SMB. Significant correlation of angiotensin converting enzyme and glycoprotein IIIa genes polymorphisms with unexplained recurrent pregnancy loss in north of Iran. Int J Reprod Biomed (Yazd, Iran). 2016;14:323–8.
57. Su M-T, Lin S-H, Chen Y-C, Kuo P-L. Genetic association studies of ACE and PAI-1 genes in women with recurrent pregnancy loss. Thromb Haemost. 2012;109:8–15. https://doi.org/10.1160/TH12-08-0584.
58. Kim JO, Lee WS, Lee BE, Jeon YJ, Kim YR, Jung SH, et al. Interleukin-1beta -511T>C genetic variant contributes to recurrent pregnancy loss risk and peripheral natural killer cell proportion. Fertil Steril. 2014;102:206–12.e5. https://doi.org/10.1016/j.fertnstert.2014.03.037.
59. Zidan HE, Rezk NA, Alnemr AAA, Moniem MIA. Interleukin-17 and leptin genes polymorphisms and their levels in relation to recurrent pregnancy loss in Egyptian females. Immunogenetics. 2015;67:665–73. https://doi.org/10.1007/s00251-015-0876-8.
60. Chen H, Yang X, Du J, Lu M. Interleukin-18 gene polymorphisms and risk of recurrent pregnancy loss: a systematic review and meta-analysis. J Obstet Gynaecol Res. 2015;41:1506–13. https://doi.org/10.1111/jog.12800.

61. Qaddourah RH, Magdoud K, Saldanha FL, Mahmood N, Mustafa FE, Mahjoub T, et al. IL-10 gene promoter and intron polymorphisms and changes in IL-10 secretion in women with idiopathic recurrent miscarriage. Hum Reprod. 2014;29:1025–34. https://doi.org/10.1093/humrep/deu043.
62. Bohiltea LC, Radoi EV. Interleukin-6 and interleukin-10 gene polymorphisms and recurrent pregnancy loss in Romanian population. Iran J Reprod Med. 2014;12:617–22.
63. Rasti Z, Nasiri M, Kohan L. The IL-6-634C/G polymorphism: a candidate genetic marker for the prediction of idiopathic recurrent pregnancy loss. Int J Reprod Biomed (Yazd, Iran). 2016;14:103–8.
64. Magdoud K, Granados Herbepin V, Messaoudi S, Hizem S, Bouafia N, Almawi WY, et al. Genetic variation in TGFB1 gene and risk of idiopathic recurrent pregnancy loss. Mol Hum Reprod. 2013;19:438–43. https://doi.org/10.1093/molehr/gat012.
65. Jang HG, Choi Y, Kim JO, Jeon YJ, Rah H, Cho SH, et al. Polymorphisms in tumor necrosis factor-alpha (-863C>A, -857C>T and +488G>A) are associated with idiopathic recurrent pregnancy loss in Korean women. Hum Immunol. 2016;77:506–11. https://doi.org/10.1016/j.humimm.2016.04.012.
66. Nasiri M, Rasti Z. CTLA-4 and IL-6 gene polymorphisms: risk factors for recurrent pregnancy loss. Hum Immunol. 2016; https://doi.org/10.1016/j.humimm.2016.07.236.
67. Saxena D, Misra MK, Parveen F, Phadke SR, Agrawal S. The transcription factor Forkhead Box P3 gene variants affect idiopathic recurrent pregnancy loss. Placenta. 2015;36:226–31. https://doi.org/10.1016/j.placenta.2014.11.014.
68. Dendana M, Hizem S, Magddoud K, Messaoudi S, Zammiti W, Nouira M, et al. Common polymorphisms in the P-selectin gene in women with recurrent spontaneous abortions. Gene. 2012;495:72–5. https://doi.org/10.1016/j.gene.2011.11.034.
69. Jung YW, Jeon YJ, Rah H, Kim JH, Shin JE, Choi DH, et al. Genetic variants in microRNA machinery genes are associated [corrected] with idiopathic recurrent pregnancy loss risk. PLoS One. 2014;9:e95803. https://doi.org/10.1371/journal.pone.0095803.
70. Hu Y, Liu C-M, Qi L, He T-Z, Shi-Guo L, Hao C-J, et al. Two common SNPs in pri-miR-125a alter the mature miRNA expression and associate with recurrent pregnancy loss in a Han-Chinese population. RNA Biol. 2011;8:861–72. https://doi.org/10.4161/rna.8.5.16034.
71. Hu Y, Huo Z-H, Liu C-M, Liu S-G, Zhang N, Yin K-L, et al. Functional study of one nucleotide mutation in pri-miR-125a coding region which related to recurrent pregnancy loss. PLoS One. 2014;9:e114781. https://doi.org/10.1371/journal.pone.0114781.
72. Su X, Hu Y, Li Y, Cao J-L, Wang X-Q, Ma X, et al. The polymorphism of rs6505162 in the MIR423 coding region and recurrent pregnancy loss. Reproduction. 2015;150:65–76. https://doi.org/10.1530/REP-15-0007.
73. Rah H, Chung KW, Ko KH, Kim ES, Kim JO, Sakong JH, et al. miR-27a and miR-449b polymorphisms associated with a risk of idiopathic recurrent pregnancy loss. PLoS One. 2017;12:e0177160. https://doi.org/10.1371/journal.pone.0177160.
74. Banerjee P, Ghosh S, Dutta M, Subramani E, Khalpada J, Roychoudhury S, et al. Identification of key contributory factors responsible for vascular dysfunction in idiopathic recurrent spontaneous miscarriage. PLoS One. 2013;8:e80940. https://doi.org/10.1371/journal.pone.0080940.
75. Gonçalves RO, Fraga LR, Santos WVB, Carvalho AFL, Veloso Cerqueira BAV, Sarno M, et al. Association between the thrombophilic polymorphisms MTHFR C677T, Factor V Leiden, and prothrombin G20210A and recurrent miscarriage in Brazilian women. Genet Mol Res. 2016;15 https://doi.org/10.4238/gmr.15038156.
76. Comba C, Bastu E, Dural O, Yasa C, Keskin G, Ozsurmeli M, et al. Role of inflammatory mediators in patients with recurrent pregnancy loss. Fertil Steril. 2015;104:1467–74.e1. https://doi.org/10.1016/j.fertnstert.2015.08.011.
77. Lee SK, Kim JY, Hur SE, Kim CJ, Na BJ, Lee M, et al. An imbalance in interleukin-17-producing T and Foxp3^{+} regulatory T cells in women with idiopathic recurrent pregnancy loss. Hum Reprod. 2011;26:2964–71. https://doi.org/10.1093/humrep/der301.
78. Rull K, Nagirnaja L, Laan M. Genetics of recurrent miscarriage: challenges, current knowledge, future directions. Front Genet. 2012;3:34. https://doi.org/10.3389/fgene.2012.00034.
79. Lyon MF. Sex chromatin and gene action in the mammalian X-chromosome. Am J Hum Genet. 1962;14:135–48.
80. Busque L, Paquette Y, Provost S, Roy D-C, Levine RL, Mollica L, et al. Skewing of X-inactivation ratios in blood cells of aging women is confirmed by independent methodologies. Blood. 2009;113:3472–4. https://doi.org/10.1182/blood-2008-12-195677.
81. Minks J, Robinson WP, Brown CJ. A skewed view of X chromosome inactivation. J Clin Invest. 2008;118:20–3. https://doi.org/10.1172/JCI34470.
82. Kurzawińska G, Barlik M, Drews K, Różycka A, Seremak-Mrozikiewicz A, Ożarowski M, et al. Coexistence of ACE (I/D) and PAI-1 (4G/5G) gene variants in recurrent miscarriage in Polish population. Ginekol Pol. 2016;87:271–6. https://doi.org/10.17772/gp/62203.
83. Mosaad YM, Abdel-Dayem Y, El-Deek BS, El-Sherbini SM. Association between HLA-E *0101 homozygosity and recurrent miscarriage in Egyptian women. Scand J Immunol. 2011;74:205–9. https://doi.org/10.1111/j.1365-3083.2011.02559.x.
84. Sui Y, Chen Q, Sun X. Association of skewed X chromosome inactivation and idiopathic recurrent spontaneous abortion: a systematic review and meta-analysis. Reprod BioMed Online. 2015;31:140–8. https://doi.org/10.1016/j.rbmo.2015.05.007.
85. Bock C, Beerman I, Lien W-H, Smith ZD, Gu H, Boyle P, et al. DNA methylation dynamics during in vivo differentiation of blood and skin stem cells. Mol Cell. 2012;47:633–47. https://doi.org/10.1016/j.molcel.2012.06.019.
86. Hanna CW, McFadden DE, Robinson WP. DNA methylation profiling of placental villi from karyotypically normal miscarriage and recurrent miscarriage. Am J Pathol. 2013;182:2276–84. https://doi.org/10.1016/j.ajpath.2013.02.021.
87. Zhu Y, Lu H, Huo Z, Ma Z, Dang J, Dang W, et al. MicroRNA-16 inhibits feto-maternal angiogenesis and causes recurrent spontaneous abortion by targeting vascular endothelial growth factor. Sci Rep. 2016;6:35536. https://doi.org/10.1038/srep35536.
88. Wang L, Tang H, Xiong Y, Tang L. Differential expression profile of long noncoding RNAs in human chorionic villi of early recurrent miscarriage. Clin Chim Acta. 2017;464:17–23. https://doi.org/10.1016/j.cca.2016.11.001.