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Genetics of recurrent miscarriage and fetal loss☆

M.H. Tur-Torres, MD [a], C. Garrido-Gimenez, MD [a], J. Alijotas-Reig, MD, PhD, MSc [b, *]

[a] Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Vall d'Hebron University Hospital, Universitat Autònoma de Barcelona, Spain
[b] Systemic Autoimmune Disease Unit, Department of Internal Medicine, Vall d'Hebron University Hospital, Professor of Medicine, Department of Medicine, Universitat Autònoma de Barcelona, Spain

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Despite years of research, miscarriage, particularly when recurrent, continues to pose a medical challenge. An embryo chromosomal error is responsible for 50–60% of recurrent cases; however, up to 30–50% remains an enigma. Successful pregnancy involves different maternal physiologic changes and certain complex interactions between the fetus and the mother by cytokines, angiogenic mediators and hormones. To date, research lines have focused on genetic and epigenetic polymorphisms related mainly to immune response and inflammatory mediators, and have yielded a significant relationship between recurrent miscarriage and immune mechanisms. Thus, unknown causes of miscarriage could be due to an immune imbalance induced by T-helper Th1/Th2/Th17 cytokines and regulatory T cells. Furthermore, these genes

Abbreviations: ACE, Angiotensin I converting enzyme; Bcl-2, B-cell lymphoma 2; CGH, comparative genomic hybridization; CYP, cytochrome P450; CTLA-4, Cytotoxic T-Lymphocyte Antigen-4; FISH, Fluorescent in situ hybridization; FVL, Von Leiden Factor; HLA-G, human leukocyte antigen-G; IFN-γ, gamma-interferon; IL, interleukins; ITGB3, integrin subunit 3; KDR, kinase insert domain receptor; miRNA, micro-RNAs; MTHFR, Methylenetetrahydrofolate reductase; NO, nitric oxide; NOs3, nitric oxide synthase; PAI-1, plasminogen activator inhibitor type 1; PGD, Preimplantation genetic diagnosis; Pten, Phosphatase and tensin homolog; qPCR, quantitative polymerase chain reaction; QFPCR, quantitative fluorescent PCR; RM, recurrent miscarriage; RPL, recurrent pregnancy loss; SM, single miscarriage; TGF-β, tumor growth factor-βeta; Th1/Th2, T-lymphocytes that deliver proinflammatory/anti-inflammatory cytokines; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor; VNTR, variable nucleotide tandem repeat.

☆ This paper has not been previously published nor is currently being under consideration for publication elsewhere.

* Corresponding author. Jaume Alijotas-Reig, MD, PhD, MSc Senior Consultant. Systemic Autoimmune Disease Unit, Department of Internal Medicine, Vall d'Hebron University Hospital, Passeig Vall d'Hebron 119-129, 08035 Barcelona, Spain. Tel.: +34 636 11 05 11.
E-mail addresses: helena.tur.torres@gmail.com (M.H. Tur-Torres), jalijotas@vhebron.net (J. Alijotas-Reig).

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and mediators have long been suspected of being blood markers for the clinical diagnosis and management of miscarriage; however, more evidence is required for them to be included in medical practice and obstetric guidelines.

Introduction

Miscarriage also referred to in the literature as pregnancy loss or abortion [1], and the term to be used herein, is the most common complication of pregnancy. It poses a clinically-frustrating and emotionally-charged challenge for patients and providers.

Miscarriage may be single (SM) or recurrent (RM). Based on the idea that separate entities could represent different pathophysiologic mechanisms leading to recurrent miscarriage, several studies have classified it according to maternal reproductive history as: primary (no pregnancies to term), secondary (series of miscarriages after a live birth) and tertiary (three non-consecutive miscarriages) [2].

Miscarriage occurs in approximately 10 to 15% of pregnancies [3,4] (4 to 5-fold higher if biochemical pregnancies are included), and is defined as the spontaneous loss of a clinically-established intrauterine pregnancy before the fetus reaches viability, i.e. up to a maximum of 24 weeks of gestation [4–6].

RM, historically defined as three or more consecutive losses before 20 weeks of gestation and affecting 1 to 3% of pregnancies [7] is currently considered by several researchers and clinicians to represented two or more losses [8]; this new definition raises the rate to 5% of sexual active couples trying to conceive [6,9].

Reproductive history is an independent predictor of future pregnancy outcome [5,6]. Accumulating evidence has suggested that the risk of a further miscarriage increases after each successive pregnancy loss, reaching 45% after three consecutive losses [5,6]. In addition, a previous live birth does not preclude a woman developing RM [5].

Genetic factors are the main cause of early miscarriage. Epidemiologically, the most common cause described is fetal chromosomal abnormalities [9,10], particularly numerical alterations, with the majority occurring *de novo* rather than being inherited [11]. Risk increases with advanced maternal age, which is also an independent risk factor for both single and recurrent miscarriages [12,13]. Other known risk factors for RM include non controlled endocrine disorders, luteal phase defects, infectious diseases, environmental factors, uterine diseases or thrombophilic disorders, particularly antiphospholipid syndrome.

Routine evaluation of a couple with RM determines a cause in less than 50% of cases [14–16], thus reflecting the heterogeneous nature of the condition. The aim of this review was to assess and synthesize the current data on genetic factors involved in miscarriage, with emphasis on novel research lines. Embryo chromosomal abnormalities, parental karyotype alterations, sperm alterations and gene polymorphisms will be discussed below. Moreover, the role of different management tools in pregnancy loss according to the literature will be proposed.

Search strategy and selection criteria

Data for this review were obtained through a comprehensive search of the literature using the relevant keywords: “recurrent miscarriage, genetic alterations, chromosome abnormalities, genetic polymorphisms” to identify articles published in English from MEDLINE, PubMed and The Cochrane Library (January 2000- December 2016). Study selection was based on existing clinical evidence. The included articles were: randomized controlled studies or meta-analyses when possible followed by prospective, matched or non-matched case-control, cohort studies and reviews. The initial search provided more than 1000 articles. Some were found in duplicate. Finally, only 78 studies regarding the

genetics of recurrent miscarriage were collected and discussed, 62 of which were included in accordance with the Editorial requirements of the Journal.

Chromosomal alterations

Abnormal embryonic karyotypes

The most common known cause of miscarriage is fetal chromosomal error, which occurs mainly before 10 weeks of pregnancy, and concerns about 50–70% of couples [6]; however, villous biopsies have demonstrated that this percentage might even reach 83% [17]. In contrast, very low rates are described in later losses: miscarriages between 12 and 22 weeks constitute approximately 4% of pregnancy outcomes, and less than 4% of these exhibit chromosomal errors [12]. This event is more frequent in SM than in RM: approximately 70% of SM samples studied showed abnormalities compared with a range of 29–50% described for RM [4,7,9,10,16,17], depending on the maternal age and number of previous miscarriages. In contrast to these findings, a recent study by Chan Wei et al. [18] of 832 miscarriage samples from Chinese women, found no statistically-significant differences in the prevalence of aneuploidy between recurrent and sporadic or single miscarriage.

Cytogenetic studies of miscarriage samples have shown that most of these abnormalities arise *de novo* in the first trimester of pregnancy. The majority are numerical modifications (86% in SM vs 90% in RM) [4], mainly autosomal trisomy, followed by polyploidy such a triploidy or tetraploidy, and monosomy X [9]. A minority of cases are caused by structural chromosomal abnormalities (6% in SM vs 8% in RM), chromosome mosaicism and other alterations (8% in SM vs 13% in RM) [4]. Single gene defects, such as those associated with cystic fibrosis or sickle cell anemia, are seldom associated with RM [14].

Autosomal trisomies are the result of maternal meiotic errors with some studies showing the most frequent chromosome involved to be 16 followed by 22, 21, 15, 18 and 2 [12]. In a case-control study with 420 specimens, Stephenson et al. [7] showed the most frequent trisomy to be 15 followed by 16, 22, 21, 14 and 13, attributing these differences to higher mean maternal age in their data set and the inclusion of cytogenetically-defined preclinical miscarriages (<6 weeks). By contrast, in pregnancy trimesters, while the commonest kind of trisomy in the first was 16 (known as "miscarriage chromosome") (Fig. 1), the most common in the second was 21 [18] (Fig. 2). Trisomy of chromosomes 19 and 1 were the rarest [12].

Polyploidy generally originate from fertilization by polyspermy or postzygotic division error and are not compatible with life [9]. The most common triploid arrangement was 69, XXY (4%) followed by 69, XXX (2.7%) [12] (Fig. 3).

Mitotic error may result in at least two cell lines (mosaicism) in the developing fetus [12]. The degree of mosaicism depends on the timing of the error e.g if the error occurs very early in the zygote, the percentages of each cell line may be equal [12].

All that abnormalities, particularly trisomies but rarely monosomy X, are strongly associated with parental age. Maternal age is widely known to be an independent risk factor for miscarriages. Current data support this theory showing losses in up to 10% of women 20–24 years of age, 51% in those 40–44 and a loss risk of 75% in women 45 or older [12,13]. However, the mechanisms involved have not been determined to date. Historically, one accepted hypothesis was the presence of diminished ovarian reserve in these women. A prospective cohort study recently conducted by Shahine et al. [14] analyzing 239 women undergoing in vitro fertilization showed that those with diminished ovarian reserve had a higher percentage of aneuploid blastocysts; however, in contrast to what was expected, more significant differences were found in patients <38 years (67% vs. 53%).

On the other hand, the role of paternal age in the pathogenesis of miscarriage continues to be an unexplored area and little research has been carried out in this field.

Chan Wei et al. [18] reported that paternal age could be involved in fetal aneuploidy, finding an association up to age of 40, after which this rate decreased. However, no other studies had found statistically-significant differences, thereby showing that more evidence is needed to confirm that association. Thus, the weight of that factor remains unclear.

Many reports have suggested that the abnormal embryonic karyotype predicts subsequent live birth [10,19]. Ogasawara et al. [10] described a cumulative live birth rate in women with miscarriage caused by an abnormal embryonic karyotype higher than that in women with recurrent miscarriage of

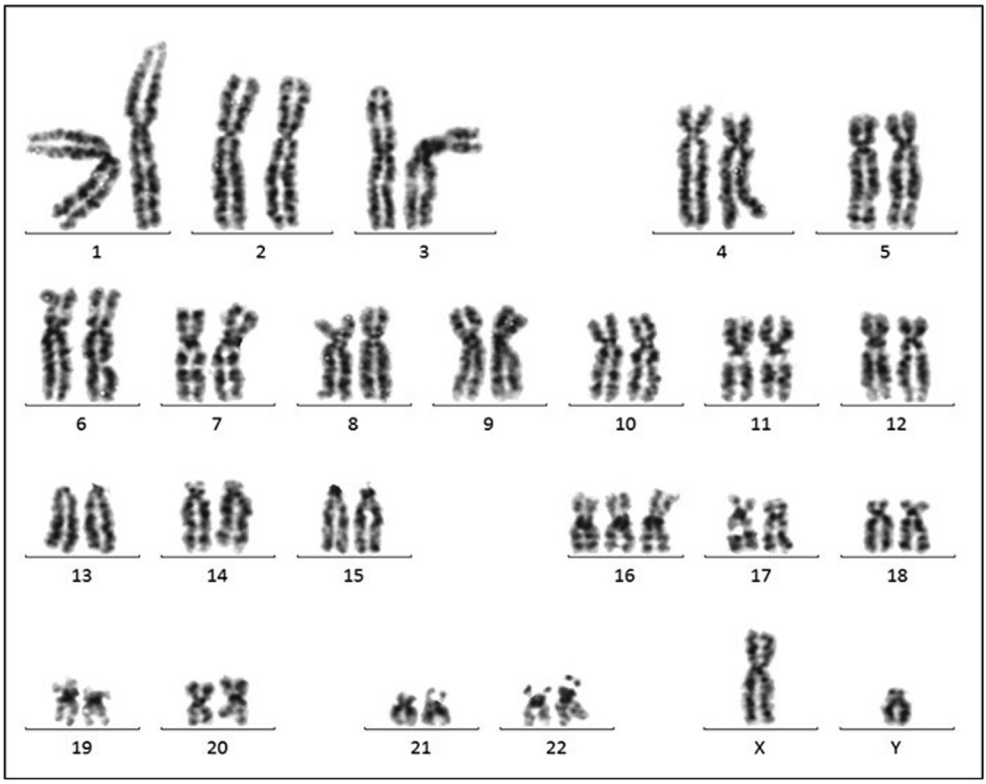

Figure 1. Abortion karyotype of trisomy 16 (47 XY, +16). Karyotype image courtesy of Carmen Mediano, PhD., Department of Genetics. Vall d'Hebron University Hospital.

truly unexplained cause (71.2% vs 52.5%). Moreover, embryonic karyotype abnormalities, whatever it were, tend to repeat in subsequent miscarriages and it is also a predictor of good prognosis for that partner, noticed for the comparison with live birth rate in both groups [10]; On the other hand, interestingly, it seems that the higher the number of miscarriages, the less probable it is that they are related to chromosomal abnormalities [3,10,17–21]. Thus, patients with RM due to the abnormal embryonic karyotype might have gene mutations associated with aneuploidy which explain their poor outcomes and the increased number of miscarriages. For example, recent findings have shown that mutations in SYCP3, a gene encoding as essential component of the synaptonemal complex, could contribute to abnormal chromosomal behavior leading to RM [10,21].

Chromosome abnormalities in either partner

The majority of miscarriages occur in chromosomally-normal parents. One partner has a structural chromosome abnormality in only 2–4% of couples with RM [4,22]. In addition, the prevalence of chromosomal aberrations appears to be independent of the number of previous miscarriages.

Various alteration types have been described: reciprocal translocation (balanced or unbalanced) caused by rearrangement of terminal parts from different chromosomes; centric fusion of two acrocentric chromosomes, known as Robertsonian translocation, being the most frequent t(13;14) and t(14;21) [23]; or inversion, in which a chromosome segment is reversed end to end. The most commonly reported human inversion is (p12;q13) [22].

Carp et al. [24], comparing chromosome abnormalities in a retrospective comparative cohort study, found that the most frequent chromosomal rearrangement to be balanced translocations (52%),

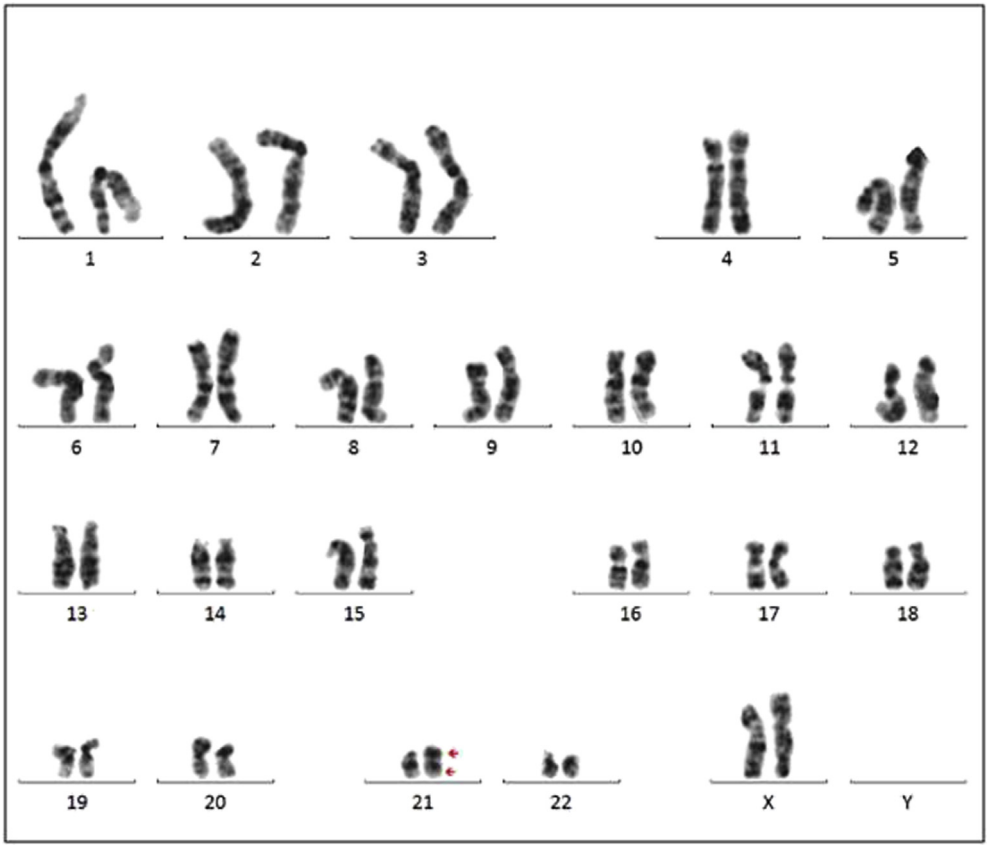

Figure 2. Rare abortion karyotype consists of trisomy 21 caused by the formation of an isochromosome 21q (46 XX, i(21)(q10;q10)). The arrows indicate the involved chromosomes. Karyotype image courtesy of Carmen Mediano, PhD., Department of Genetics. Vall d'Hebron University Hospital.

followed by inversions (26%), with a significantly greater prevalence in the male partner. Mosaics were found at a frequency of 21%, with a significantly greater prevalence in the female partner.

Although carriers of balanced alterations are usually phenotypically normal and have a demonstrated possibility of giving birth, up to 50–70% of their gametes and hence embryos are unbalanced owing to of errors that occurred during segregation at meiosis [6]. Thus, their pregnancies are at increased risk of miscarriage and may result in a live birth with multiple congenital malformations and/or mental disability secondary to an unbalanced chromosomal arrangement [5]. The reproductive risk seems to depend on the type of rearrangement, the size and genetic content of the rearranged chromosomal segments, and the mode of ascertainment. However, there was no significant distinctive effect if the aberration was maternally- or paternally-derived [24].

Ogasawara et al. [25], who analyzed the karyotypes of 1284 couples, suggested that the presence of parental karyotype aberrations defines a group of high risk for miscarriages, since between 61% and 72% of their patients had subsequent miscarriages.

However, the studies by Goddijn et al. [26] and a case-control study by Franssen et al. [27] showed that the proportion of couples giving birth to one or more healthy newborns was similar in the various types of abnormalities, with no significant differences in birth rates for any kind of alterations. In the Carp et al. [24] study outcomes were 83% for reciprocal translocations, 82% for Robertsonian translocations, 78% for inversions and 93% for other abnormalities. Furthermore, analyzing the reproductive

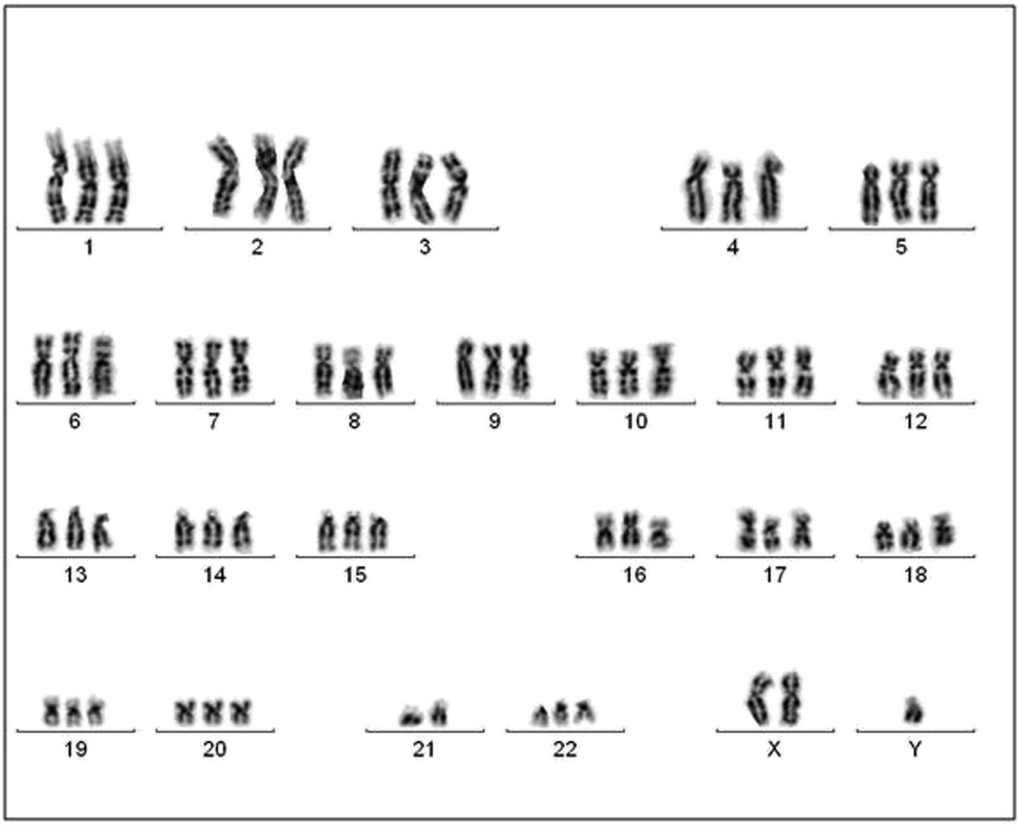

Figure 3. Abortion karyotype of triploidy with disomy 21 (68 XXY, -21). Karyotype image courtesy of Carmen Mediano, PhD., Department of Genetics. Vall d'Hebron University Hospital.

outcome after chromosome analysis in couples with RM, they reported that couples whose carrier status was ascertained after two or more losses had a low risk of viable offspring with unbalanced chromosomal abnormalities, and their chances of having a healthy child were as good as non-carrier couples, despite a higher risk of miscarriage [27].

It seems more likely that the prognosis for a subsequent live birth depends more on factors such as the number of previous miscarriages, maternal age, karyotype of the previous miscarriage, and primary or secondary aborted status rather than parental karyotype [11].

Sperm implication in chromosomal abnormalities

As mentioned previously, the majority of chromosome abnormalities arise *de novo* from random errors produced essentially during three development stages: gametogenesis, fertilization and embryonic development [28]. The current knowledge shows that those alterations are mostly derived from non-disjunction errors during the first meiotic division of the oocyte, with a significant association with advanced maternal age.

On the other hand, the male role in those errors remain uncertain, and sperm studies have demonstrated that paternal meiotic errors also occur and have the potential to cause abnormal fertilization. Sperm quality has been associated with the embryo's ability to reach the blastocyst stage and progress to implantation [29]. A meta-analysis conducted in 2012 showed a significant increase of miscarriage in patients with major DNA damage compared to those with minor DNA damage [30].

Although no significant changes in sperm morphology, mean count and motility had been found in the past, a recent review reported a chromosomal abnormality frequency of 15.2% in men with azoospermia and in 2.3% of nonazoospermic men [29].

After analyzing 12 couples with RM, Rubio et al. [28] reported a significant increase in disomy frequency for sex chromosomes and diploidy rates in sperm samples from RM couples compared to those from internal controls (0.84% vs 0.37%). This was confirmed by Carrell et al. [31] (2.77% vs 1.19%), indicating that this may be an important etiologic factor in RM. Agarwal et al. [32] suggested that microdeletions of the azoospermia factor located in the long arm of chromosome Y, and which are essential for normal spermatogenesis, may have a direct effect on the early prophase of mitosis decreasing the normal pairing rate in the pachytene stage of spermatocytes. This pairing failure may increase chromosomal abnormalities and could be related to RM.

In addition, a case-control study based in sperm samples of 11 partners affected with unexplained RM, revealed a statistically-significant increase in meiotic errors involving chromosome 16 which contributed to increased sperm disomy in more than 60% of their patients [16]. Thus, these data suggest that among paternal meiotic errors the non-disjunction of chromosome 16 might exert similar relative influence on fetal aneuploidy compared with maternal chromosome 16 disomy [16].

The association of sperm quality with recurrent pregnancy loss emphasizes the importance of evaluating the male factor. Several different tests are available, but no consensus has yet been reached as to which tests are more predictive [29].

Genetic disorders

Certain genetic mutations, such as autosomal-dominant disorders leading to myotonic dystrophy, may predispose patients to infertility or even RM [33]. Other presumed autosomal-dominant disorders associated with RM include lethal skeletal dysplasia, connective tissue disorders such as Marfan syndrome, Ehler-Danlos or Pseudoxanthoma elasticum, or hematologic abnormalities including fibrinogen alterations, factor XIII deficiency or sickle cell anemia [15,33].

Since 1990s, many genetic polymorphisms have been proposed as being implicated in pathogenesis of RM. It has been suggested that biologic processes for maintaining the pregnancy stability are mediated by the expression of a series of different genes related to inflammatory mediators, placental function regulators, thrombogenic factors and sex hormone receptors [34]. For this reason, research lines had focused on principally seeking these related candidate genes. However, the results of these studies were usually inconsistent, especially when they were conducted in different populations.

In 2004, Baek at al [35] described 30 genes showing different levels of expression between normal and RM patients which were involved in immunity, angiogenesis and apoptosis pathways. Later, other research groups also identified a large number of genes that are expressed aberrantly in pregnancy failure [1,36]. Xiaonhan et al. [34] recently published a large meta-analysis in which 53 polymorphisms of 37 genes were shown to be associated with RM. However, Pereza et al. [1] reported in their meta-analysis that the results of all associations were modest, and the pathophysiologic mechanisms of how specific genetic variants might contribute to RM remain largely unexplored.

Of all RM etiologic factors, scientists are currently more interested in the field of reproductive immunology [37]. T lymphocyte plays a central role in cell-mediated immunity and two main subsets of them are described depending on the presence of cell surface molecules: CD4 and CD8. T lymphocytes expressing CD4, also known as helper T cells (Th) can be further subdivided into Th1 (pro-inflammatory response) and Th2 (anti-inflammatory response). Since a close relationship between RM and immune mechanisms was observed in some studies, suggesting that the unknown causes of miscarriage could be explained by immune imbalance induced by Th1/Th2 cytokines [38] towards mounting Th1 response [5].

Immune response (Table 1)

Pro-inflammatory cytokines are known to exert an adverse effect on pregnancy; many of them, which are mostly regulated by Th1/Th2 balance, have been reported to be associated with RM [38,39].

Table 1
Immune response polymorphisms related to recurrent miscarriage.

HLA-G	Nazila Alizadeh et al. (2016)
	Xiaohan et al. (2016)
	Arjmand et al. (2016)
TGF-β	Xiaohan et al. (2016)
TNF-α	Choi et al. (2008)
	Hiu-Hiu et al. (2016)
	Xiaohan et al. (2016)
	Zhang et al. (2016)
IL-6	Rasti et al. (2016)
INF-γ	Xiaohan et al. (2016)
	Young Ho et al. (2015)
IL-1-β	Suzomi et al. (2010)
IL-1R	Xiaohan et al. (2016)
IL-4	Xiaohan et al. (2016)
IL-10	
IL-17	
IL-18	
IL-23	Abdollahi et al. (2014)
	Xiaohan et al. (2016)
IL-33	Jun et al. (2016)
	Xiaohan et al. (2016)
CTLA-4	Rasti et al. (2016)

HLA-G: human leukocyte antigen-G; TGF-β: tumor growth factor-βeta; TNF-α: tumor necrosis factor alpha; IFN-γ: gamma-interferon; IL: interleukins; CTLA-4: Cytotoxic T-Lymphocyte Antigen-4.

During pregnancy, the normally-dominant Th-1 inflammatory immune response switches to a Th2 cytokine profile, thereby permitting induction of maternal immune tolerance in the allogenic fetus and assuring successful implantation and reproductive outcomes. If the Th1 response prevails beyond Th2 as a result of an immune imbalance induced by cytokines, the risk of RM is increased.

Some examples of cytokine roles such as interleukin (IL) -4, IL-6 and IL-10 may promote embryo development and placentation, whereas tumor necrosis factor (TNF-α) and gamma-interferon (IFN-γ) inhibit trophoblast growth and differentiation by acting as embryo toxic cytokines [38].

Among genetic variants, human leukocyte antigen-G (HLA-G) (rs66554220), the most dominant HLA antigen in blastocysts and trophoblastic tissue, IL-6 (rs1800795) and TNF (α-308G/A, rs1800629; OR 0.75) are the three most extensively studied polymorphisms, and significant differences were found with RM in the Xiaohan et al. meta-analysis [34]. Two more recent meta-analyses published, also found a statistically-significant association between serum TNF-α levels and RM [38,40], possibly promoting decomposition and apoptosis in the chorion and decidua and thrombotic events in the placenta, thereby raising the risk of embryo loss [41]. Specifically, the TNF-α-308G/A polymorphism was found to be a risk factor; however, no correlation was observed between the -238G/A polymorphism and susceptibility to RM [40]. The opposite is true for IL-6: a significant association was also found between IL-6-174 G/C, IL-6-634 G/C and low serum IFN-γ +874 A/T concentrations and susceptibility to RM, particularly in a non-Caucasian population [34,42,43].

Significant results were also observed for polymorphisms of IL-1β [21] and its receptor (IL-1R), IL-17 and IL-18 with high rates in serum (pro-inflammatory cytokines) in contrast to IL-4, IL-10, IL-23 [44], IL-33 [45] (only demonstrated in a Chinese population) and reduced serum levels of transforming growth factor-beta (TGF-β) [34].

Moreover, it has been hypothesized that the cytotoxic T-Lymphocyte antigen-4 (CTLA-4) +49 G allele may act as a dominant allele, lowering the risk of RM, especially among Iranian and Indian women [46]; however, this result was highly influenced by differences among populations, and more evidence is therefore required to confirm this hypothesis.

Thrombophilia (Table 2)

Genetic polymorphisms directly or indirectly related to coagulation or fibrinolysis processes have also been widely studied in association with late RM or fetal loss, since they act as disruptors of homeostatic balance in pregnancy [47].

The most commonly studied factors are Von Leiden Factor (FVL) (rs6025), FII (rs1799963) and FXIII (G103T) polymorphisms, with a significant association being found between them and prothrombin accumulation, which may explain their connection to the thrombotic events and RM studied mainly in a Caucasian population [34]. In contrast, allele mutated factor VII (Gln353 allele of the Arg353Gln and -122C allele of the -122T>C) was related to low rates of miscarriage and considered as a possible protective mutation by the results of a novel study, especially in women with three or more pregnancy losses [48].

Methylenetetrahydrofolate reductase (MTHFR) polymorphisms (rs1801131 and rs1801133) are a further two well-known genetic variants. Mutations in this gene may inhibit the production of tetrahydrofolate reductase which results in hyperhomocytenemia and thrombophilia [33,34]. In addition, a significant association has also been o detected between RM and the 4G allele of the plasminogen activator inhibitor type 1 (PAI-1) rs1799889 polymorphism, which may inhibit the effects of fibrinolysis by increasing the level of PAI-1, resulting in excessive thrombosis and contributing to the pathogenesis of RM [34]. No association has been confirmed in meta-analyses between the -675 4G/5G gene polymorphism and the risk of RM, despite differences found separately in previously studies [36].

Angiotensin I-converting enzyme (ACE) plays a vital role in maintaining homeostasis; historically, some studies suggested that the ACE intron 16 I/D polymorphism, resulted in to an elevated ACE levels and substantially more activated angiotensin-converting enzymes, thereby increasing predisposition towards thrombosis and RM [49]. However, the Xiaohan et al. [34] meta-analysis failed to reveal a significant association between the ACE insertion/deletion variant and RM in any comparative models in the overall analyses.

PLA2 polymorphisms of integrin subunit 3 (ITGB3) were also related to RM owing to the promotion of thromboembolic complications of pregnancy [50].

Placental function (Table 3)

Placental angiogenesis involves the formation of new branches from pre-existing vessels and the remodeling of an existing vascular network through three main processes: initiation, proliferation-invasion and maturation-differentiation. Abnormalities in these steps are considered one of the leading causes of RM [51].

Table 2
Thrombophilia polymorphisms related to recurrent miscarriage.

FII	Xiaohan et al. (2016)
FVL	Chaithra et al. (2011)
	Xiaohan et al. (2016)
FVII	Barlik et al. (2016)
FXIII	Xiaohan et al. (2016)
MTHFR	Chaithra et al. (2011)
	Xiaohan et al. (2016)
PAI-1	Torabi et al. (2012)
ACE	Wiwanitkit et al. (2004)
ITGB3	Ruzzi et al. (2005)

FII: factor II, prothrombin; FVL: Von Leiden Factor; FVII: factor VII, proconvertin; FXIII: factor XIII, fibrin stabilizing factor; MTHFR: Methylenetetrahydrofolate reductase; PAI-1: plasminogen activator inhibitor type 1; ACE: Angiotensin I converting enzyme; ITGB3: integrin subunit 3.

Table 3
Placental function polymorphisms related to recurrent miscarriage.

VEGF	Xu et al. (2015)
NOs3	Xiaohan et al. (2016)
KDR	Xiaohan et al. (2016)
miRNAs	Zhu et al. (2016)
	Wang et al. (2016)
p53	Daher et al. (2012)
	Xiaohan et al. (2016)

VEGF: vascular endothelial growth factor; NOs3: nitric oxide synthase; KDR: kinase insert domain receptor; miRNA: micro-RNAs.

Vascular endothelial growth factor (VEGF) is a signal protein that stimulates implantation and development of the embryo, placental angiogenesis and the growth of maternal and fetal blood vessels in the uterus. Moreover, it is a well-known mediator of nitric oxide (NO), an indispensable modulator of vascular tone which induces vasodilatation. Insufficient NO production can impair placental perfusion and endometrial receptivity, which may be associated with the pregnancy loss.

A meta-analysis conducted by Xu et al. [52] showed that -1154G/A (rs157036), +936C/T (rs3025039), -634G/C (rs2010963) and -538T/C (rs3025020) polymorphisms of the VEGFA gene, associated with decreased serum levels of the VEGF protein, increase individual RM susceptibility. Both, rs3025039 and rs1570360 polymorphisms also showed significant associations in Caucasian populations in the Xiaohan et al. meta-analysis [34]. No statistically-significant association with -2578C/A (rs699947) was observed [52]. VEGF receptor 2 gene (KDR) polymorphisms have also been related to RM [34]. In contrast, the 27 bp of the variable nucleotide tandem repeat (VNTR) polymorphism in intron 4 is the over-dominant model and the rs1799983 polymorphism of endothelial nitric oxide synthase (NOs3) has been related to RM in single studies; however, systematic reviews and meta-analyses did not confirm this association [34,53].

Micro-RNAs (miRNA) are RNA species that induce post-transcriptional gene silencing and mediate translational repression through binding to target mRNA, leading to subsequent mRNA degradation. The placenta production of a large number of miRNA involved in its development has been demonstrated. Zhu et al. [51] recently suggested that miRNA-16 regulates placental angiogenesis and development by targeting VEGF expression. Higher miRNA-16 expression was found in villi and decidua of an RM patient, apparently suppressing VEGF expression. Thus, VEGF may act as a potential target gene of miRNA-16, being inversely correlated with miRNA-16 expression, and increase the risk of miscarriage.

A recent case-control study conducted by Wang et al. [54], including 18 patients with RM and 15 normal pregnant women, suggested that the lower expression of has-miR-1 and -372 might be involved in the progression of RM by targeting two apoptosis molecules of the tumor protein 53 (p53) signaling pathway: B-cell lymphoma 2 (Bcl-2) or phosphatase and tensin homolog (Pten), respectively. In contrast, in the decidua and villi of RM patients, expressions of has-miR-516a-5p, -517a-3p, -519a-3p and -519d, and has-miR-100 and -146a-5p were significantly up-regulated compared to normal pregnancy.

As a classical regulator of apoptosis and genomic stability, p53 is considered to be an essential regulating factor in embryonic development. The rs1042522 polymorphism, also known as an Arg72Pro variant of p53, resulting in a lack of its activity, is suspected of being associated with RM [34,53].

Hormonal and detoxification system (Table 4)

Some environmental and lifestyle factors are known to increase oxidative stress through their activation and elimination by members of detoxification systems. Mostly represented by the cytochrome P450 (CYP) family of enzymes, all these processes produce reactive oxygen species [55]. Among

Table 4
Hormonal and detoxification system polymorphisms related to recurrent miscarriage.

CYP family* (CYP17, CYP1A1, CYP2D6)	Chaithra et al. (2011) Suryanarayana et al. (2004) Xiaohan et al. (2016)
Progesterone	Daher et al. (2012)

*CYP: cytochrome P450.

CYP family members, components of which are expressed in the placenta during the first trimester, CYP17 rs743542, CYP1A1 rs1048943 and CYP2D6 rs3892097 polymorphisms were shown to be correlated with the risk of RM [34]. The first has been reported to be related to altered serum levels of both androgens and estrogens, and this may result in the disruption of pregnancy processes; as well an increased risk of miscarriage with maternal smoking and alcohol consumption [33]. Second and third are crucial modulators of the detoxification system and could protect the utero-placental environment from being affected by overwhelming oxidative stress.

The role of hormones in reproductive outcomes is widely known. Progesterone is the hormone most clearly associated with maternal adaptation to pregnancy; estrogen is also important for reproductive success [53]. It has been suggested that different functional polymorphisms in the gene encoding progesterone and estrogen receptor [56] might have a relationship with RM; however, studies to date have been contradictory and further research is required to analyze this relationship and confirm or refute this hypothesis.

Other genetic polymorphisms (Table 5)

Some special genetic polymorphisms are also described in the literature. Annexin A5, also termed the placental anticoagulant protein, is a ubiquitously expressed phospholipid-binding annexin [57]. It forms a protective antithrombotic shield at the surface of placental syncytiotrophoblasts and is an essential component of placental integrity [58]. Disruption of this layer and reduced levels of annexin A5 protein on the placental villi, led by polymorphisms in the core promoter of the annexin A5 gene (ANXA5, 4q27) termed M2 haplotype, has been associated with increased risk of RM in central Europe and Asia [57].

Management of genetic anomalies

The gold standard test used to identify fetal chromosomal abnormalities is G-banding conventional karyotyping [12,59], defined as the morphologic characterization of the chromosomal complement of an individual including number, form and size of the chromosomes. It is one of the whole genome techniques. However, it may be hampered by maternal contamination, culture failure or overgrowth and poor quality of chromosomal preparations [4], and can only detect numerical or structural chromosomal changes resulting from the loss or gain of upwards of 5 megabases of genetic material [59].

Fluorescent in situ hybridization (FISH), comparative genomic hybridization (CGH), array-based CGH, single nucleotide polymorphism detection, quantitative polymerase chain reaction (qPCR) and quantitative fluorescent PCR (QF-PCR) are molecular techniques that are often said to deal with a part of the limitations of conventional karyotyping [4]. They are able to detect microdeletions and microduplications sizing upwards to 500 pairbases that cannot be detected by traditional techniques, and

Table 5
Other polymorphisms related to recurrent miscarriage.

Annexin A5	Nagirnaja et al. (2015) Rand JH (2000)

may contribute to detecting abnormalities in cases of culture failure or maternal contamination. These techniques can be subdivided in to whole genome techniques (CGH, array-CGH) and chromosome-specific techniques (FISH, qPCR, QF-PCR). A recent systematic review by Saldarriaga et al. [59] concluded that CGH exhibits higher sensitivity with similar specificity what does karyotyping, and clinical heterogeneity has to be considered, thereby, confirming the data from previous meta-analyses and reviews [4].

Although the value of knowledge on the prevalence of cytogenetic abnormalities in miscarriage samples is undisputed, current data differ as to the usefulness of molecular techniques and karyotyping of miscarriage samples in routine clinical practice.

After analyzing differences between techniques in prenatal diagnosis, some studies comparing different chromosomal alteration analysis techniques reported diagnostic frequencies of 2.5–4.2% with karyotyping, whereas frequencies of 5.3–15% were reported with molecular tools [59,60]. By contrast, in a systematic review by Van den Berg et al. [4], no more chromosome abnormalities were detected by molecular techniques, and costs were higher compared with the gold standard, suggesting that molecular techniques could be useful as a complementary tool, but not be used instead of karyotyping as opposite to the Chu et al. [61] study which suggested that molecular techniques, particularly CGH-array, could be considered one of the first diagnostic tests instead of the conventional ones. Hardy et al. [12] summarized that a combined approach using conventional and molecular methods would elucidate the cause of miscarriage in almost all samples and, in a clinical setting, this would be optimum. Thus, there is no clear guidance for clinical decision-making, whereas a genetic test result may provide information for the couple in question.

Preimplantation genetic diagnosis (PGD) is the screening of embryos at the cleavage stage in order to select and transfer the desired embryo, and is mainly performed in fetus transfer after in-vitro fertilization. It is widely performed for couples with RMs, and has been proposed as a treatment option for translocation carriers [5]. However, information on whether PGD can improve success rates in parental chromosome abnormality carriers is limited. Lalioti et al., [62] suggested that PGD reduces the miscarriage rate in couples with RM due to a preexisting chromosomal imbalance in one of the parents or in patients over 35 years of age. In contrast, Raj et al. [6] showed that aneuploidy screening and the replacement of chromosomally-normal embryos does not improve the live birth rate.

In conclusion, systematic reviews showed that there is no conclusive evidence to support prenatal genetic screening for unexplained RM, or for a structural chromosome abnormality in couples ascertained for recurrent miscarriage. Furthermore, owing to the lack of evidence, assisted conception with pre-implantation genetic screening as a treatment of RM is not recommended [29].

Summary

Recurrent miscarriage is difficult to manage owing to the lack of knowledge on molecular mechanisms of its pathogenesis. Fetal chromosomal abnormalities are a widely known cause of miscarriage and have been defined as the main cause of both single and recurrent miscarriage. In their diagnosis, current data differ as to the usefulness of karyotyping and molecular techniques in routine clinical practice, leaving the decision to use or not use those tools to the individual clinician.

In an attempt to identify novel potential targets and non-invasive biomarkers for the clinical diagnosis and treatment of RM, genetic and genomic studies have been conducted to establish the expression of certain gene polymorphisms as the cause of RM. Genetic variants of some molecules appear to be correlated with this increased risk, suggesting that over-active inflammatory responses (IFN-γ, TNF-α, IL-1β, IL-1R, IL-6, IL-17, IL-23), inherited thrombophilia, acquired hyper-coagulative states i.e antiphospholipid antibody syndrome, abnormal placental function (NOS3, KDR, TP53, VEGFA), and metabolic, hormonal and detoxification system anomalies (CYP17, CYP1A1, CYP2D6) may contribute to the pathogenesis of RM, as well as genetic polymorphisms of ANXA5. Although significant associations have been found between many genetic variants and RM, further functional research is needed to establish their role as blood biomarkers and their introduction into routine clinical practice.

Practice points

- The etiology of miscarriage is unknown in approximately 50% of couples.
- The most common cause of miscarriage, particularly recurrent early miscarriage, is chromosomal abnormality.
- Chromosomal alterations mostly arise *de novo* because of non-disjunction errors during the first meiotic division of the oocyte, and are related to advanced maternal age. Therefore, the majority of miscarriages occur in chromosomally-normal parents.
- Some genetic polymorphisms, performing different roles in the immunologic response, thrombophilia and hypercoagulative state, abnormal placental function and metabolic regulation disorders correlated with an increased risk or RM.
- Molecular techniques for characterizing aborted embryo chromosomes could help to deal with conventional karyotype limitations.
- Couples with a history of RM have a 60-75% chance of a successful pregnancy next time with no therapeutic intervention.

Research agenda

- Role of paternal age and sperm alterations in fetal chromosome abnormalities.
- Effectiveness of the use of immune, thrombotic, placentation and metabolic factors as non-invasive blood markers of susceptibility to RM, and of genetic polymorphisms studied.
- Effectiveness of karyotyping and molecular technique use in miscarriage samples and their involvement in reproductive outcomes.
- Effectiveness of PGD (mainly in *in vitro* fertilization) in couples with RM.

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Conflict of interest statement

The authors also state that they do not have any commercial, financial, or any other type of interest that may influence the drawing up and the results of this.

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