A Systematic Review to Guide Future Efforts in the Determination of Genetic Causes of Pregnancy Loss

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Background: Pregnancy loss is the most common obstetric complication occurring in almost 30% of conceptions overall and in 12–14% of clinically recognized pregnancies. Pregnancy loss has strong genetic underpinnings, and despite this consensus, our understanding of its genetic causes remains limited. We conducted a systematic review of genetic factors in pregnancy loss to identify strategies to guide future research.

Methods: To synthesize data from population-based association studies on genetics of pregnancy loss, we searched PubMed for relevant articles published between 01/01/2000-01/01/2020. We excluded review articles, case studies, studies with limited sample sizes to detect associations (N < 4), descriptive studies, commentaries, and studies with non-genetic etiologies. Studies were classified based on developmental periods in gestation to synthesize data across various developmental epochs.

Results: Our search yielded 580 potential titles with 107 (18%) eligible after title/abstract review. Of these, 54 (50%) were selected for systematic review after full-text review. These studies examined either early pregnancy loss (n = 9 [17%]), pregnancy loss > 20 weeks’ gestation (n = 10 [18%]), recurrent pregnancy loss (n = 32 [59%]), unclassified pregnancy loss (n = 3 [4%]) as their primary outcomes. Multiple genetic pathways that are essential for embryonic/fetal survival as well as human development were identified.

Conclusion: Several genetic pathways may play a role in pregnancy loss across developmental periods in gestation. Systematic evaluation of pregnancy loss across developmental epochs, utilizing whole genome sequencing in families may further elucidate causal genetic mechanisms and identify other pathways critical for embryonic/fetal survival.

Keywords: early pregnancy loss, recurrent pregnancy loss, stillbirth, fetal death, genetics
Pregnancy loss is the most common obstetric complication occurring in about 30% of conceptions (1). Approximately 10–28% of all clinically recognized pregnancies result in losses (2); of these, most occur prior to the second trimester. In the United States, losses after 20 weeks’ gestation occur 1 in 160 pregnancies (3). The risk of pregnancy loss increases with a previous loss (4), suggesting that genetics may play a role in families experiencing recurrent losses. Pregnancy loss recurs in about 1–2% of couples who are trying to conceive (5), and about 25% of women attempting pregnancy experience at least one loss (6). Approximately, 50% of recurrent pregnancy loss (RPL) cases are idiopathic (i.e., without any known etiologies) (7).

Genetic abnormalities (chromosomal and single-gene disorders) in the conceptus are an established etiology of pregnancy loss (8). Fetal or placental karyotype analyses allow detection of aneuploidy (chromosomal abnormalities) in 55% of first trimester losses, 35% of second trimester losses, and 7% of losses >20 weeks’ gestation (9), confirming the higher rate of genetic factors contributing to losses in earlier gestation (10). However, genetic causes of losses >20 weeks’ gestation may not be identified by karyotype (3). Recent studies in a large cohort of losses >20 weeks’ gestation identified aneuploidy or pathogenic copy number changes as genetic causes of losses >20 weeks’ gestation in 44 (9.5%) cases using chromosomal microarray analysis (3) and single-gene pathogenic variants in 13 genes (7 previously identified and 6 strong candidates) causing 15 (6.1%) losses >20 weeks’ gestation using whole exome sequencing (WES) (11). Although findings from these studies may guide future research into mechanisms of pregnancy loss, they do not adequately facilitate clinical efforts to genetically screen losses across different developmental epochs (10, 12).

Studies that examine DNA from products of conception, as well as the parent-offspring trio (maternal, paternal, and fetal) samples, will be critical to identify causal variants and clinically significant genes. In addition, studies that identify pathways that are essential for normal and abnormal pregnancy may facilitate the discovery of novel therapeutic targets to improve pregnancy outcomes.

With the advent of next-generation sequencing (NGS), studies in the past 20 years have identified genetic pathways that are essential for in utero survival. In particular, some studies have shown increased likelihood of a genetic cause in early pregnancy (13), while challenges (e.g., accessibility, maternal cell contamination) remain when assessing biospecimen in products of conception from early losses. Furthermore, inconsistencies in categorizing pregnancy loss by gestational age have been noted by others (14). Using suggested standardized definitions of pregnancy loss, we underscore the importance of categorizing losses with regard to gestational age and developmental stage at the time of loss in future studies (10, 14). We conducted a systematic review to highlight genetic/multi-omic studies of pregnancy loss conducted between 2000 and 2020 and discussed key strategies to guide future relevant research efforts. Studies were classified based on developmental periods in gestation to synthesize data across various developmental epochs, allow classification by stage and etiology of loss (14) and identify common pathways (15).

HIGHLIGHTS

- The etiologies of PL and its genetic causes are poorly understood.
- Limited number of studies identified genetic pathways essential for PL.
- Genetic pathways are essential for embryonic/fetal survival and human development.
- Future research strategies require systematic evaluation of PL in families.

INTRODUCTION

Pregnancy loss is the most common obstetric complication occurring in about 30% of conceptions (1). Approximately 10–28% of all clinically recognized pregnancies result in losses (2); of these, most occur prior to the second trimester. In the United States, losses after 20 weeks’ gestation occur 1 in 160 pregnancies (3). The risk of pregnancy loss increases with a previous loss (4), suggesting that genetics may play a role in families experiencing recurrent losses. Pregnancy loss recurs in about 1–2% of couples who are trying to conceive (5), and about 25% of women attempting pregnancy experience at least one loss (6). Approximately, 50% of recurrent pregnancy loss (RPL) cases are idiopathic (i.e., without any known etiologies) (7).

Genetic abnormalities (chromosomal and single-gene disorders) in the conceptus are an established etiology of pregnancy loss (8). Fetal or placental karyotype analyses allow detection of aneuploidy (chromosomal abnormalities) in 55% of first trimester losses, 35% of second trimester losses, and 7% of losses >20 weeks’ gestation (9), confirming the higher rate of genetic factors contributing to losses in earlier gestation (10). However, genetic causes of losses >20 weeks’ gestation may not be identified by karyotype (3). Recent studies in a large cohort of losses >20 weeks’ gestation identified aneuploidy or pathogenic copy number changes as genetic causes of losses >20 weeks’ gestation in 44 (9.5%) cases using chromosomal microarray analysis (3) and single-gene pathogenic variants in 13 genes (7 previously identified and 6 strong candidates) causing 15 (6.1%) losses >20 weeks’ gestation using whole exome sequencing (WES) (11). Although findings from these studies may guide future research into mechanisms of pregnancy loss, they do not adequately facilitate clinical efforts to genetically screen losses across different developmental epochs (10, 12).

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With the advent of next-generation sequencing (NGS), studies in the past 20 years have identified genetic pathways that are essential for in utero survival. In particular, some studies have shown increased likelihood of a genetic cause in early pregnancy (13), while challenges (e.g., accessibility, maternal cell contamination) remain when assessing biospecimen in products of conception from early losses. Furthermore, inconsistencies in categorizing pregnancy loss by gestational age have been noted by others (14). Using suggested standardized definitions of pregnancy loss, we underscore the importance of categorizing losses with regard to gestational age and developmental stage at the time of loss in future studies (10, 14). We conducted a systematic review to highlight genetic/multi-omic studies of pregnancy loss conducted between 2000 and 2020 and discussed key strategies to guide future relevant research efforts. Studies were classified based on developmental periods in gestation to synthesize data across various developmental epochs, allow classification by stage and etiology of loss (14) and identify common pathways (15).
e.g., genes essential for embryonic lethality and functional genes essential for human development (e.g., cardiomyopathy).

**Study Summarization**

In this systematic review, we summarized the studies according to PubMed ID, first author last name and initial, year of publication, pregnancy loss outcome, predictor(s), method of assessment or study design, sample size, and tissue. We provide studies that identified candidate genes with functional pathways. For studies that did not report specific pathways, we conducted an Online Mendelian Inheritance in Man (OMIM) search to identify the roles of the reported genes in disease or functional pathways. Finally, we focused our discussion toward studies that report findings based on genetic factors that are likely causal (e.g., single-gene, autosomal and/or recessive de novo or inherited mutations, “intolerome,” copy number variations [CNVs], single nucleotide polymorphisms [SNPs]) (13). We summarized multi-omic studies, e.g., studies based on proteins and methylated genes that have different mechanisms than single-gene mutations or CNVs. The literature search was cross-examined by Authors. All conflicts were discussed and resolved before proceeding to systematic review.

**Systematic Review**

The PRISMA 2020 checklist was utilized to ensure the manuscript conformed to the systematic review definition. Of note, this study has not been registered with a specific review protocol. There are no randomized clinical trials on genetics of pregnancy loss. Risk of bias was not assessed, principle summary measures were not utilized, and synthesis of data for a meta-analysis was not performed.

**RESULTS**

**Screened Studies Selected for Systematic Review**

Our search yielded 580 potential records. The PRISMA flow diagram is provided in Figure 1. After title and abstract review, 38 records were excluded after additional filters for articles that are not full text, based on non-human studies, and not identified as English articles. After title/abstract review, additional 446 records were excluded because they were either descriptive/commentaries, studies with small sample size (n < 4), qualitative studies, systematic or comprehensive reviews, studies based on infertility and non-spontaneous abortion, or ambiguous with critical information missing. After full-text review, 53 full-text articles that were based on non-genetic factors associated with pregnancy were excluded. In the present study, we included 54 studies that reported findings based on genetic/multi-omic etiologies involved in pregnancy loss.

**Genetic Factors Associated With EPL**

Nine studies (17%) examined genetic factors in relation to EPL (Table 1). Most of the studies identified dysregulated miRNAs, epigenetic regulators which may have important role in placental development and function. The largest of these, with sample size reaching 105 participants, showed that miR-378a-3p is downregulated in early pregnancy loss (n = 50) compared with normal (n = 55) decidua (24). Hosseini et al. detected other dysregulated microRNAs (e.g., miR-135a) in maternal plasma and villous cells of women (n = 16) who had EPL, but the comparison group were women (n = 8) who underwent abortions (23). Using endocervical specimens collected prior to EPL (n = 20), altered protein expression patterns of extra villous trophoblast (EVT), which plays a role in proper implantation and placentation, were detected in cases compared to controls (21). The authors’ ability to obtain EVT cells early from ongoing pregnancies and determine the eventual pregnancy loss occurrences may have allowed opportunities to discover novel biomarkers through global analytic approaches (21).

**Genetic Factors Associated With Losses > 20 Weeks’ Gestation**

Since a standardized definition of stillbirth has not been agreed upon, studies examining loss >20 weeks’ gestation were lumped together and classified based on their specific cutoffs. One study examined self-reported miscarriage or stillbirth as the primary outcome over a broad range of gestational ages and 10 studies (18%) examined losses >20 weeks’ gestation as the primary outcome (Table 2). Of these 10 studies, cutoffs of 20, 22, 23, 24, and 32 weeks were utilized (Table 2). Seven studies examined the associations of genes involved in maternal thrombophilia with losses >20 weeks’ gestation. The largest of these, with sample size reaching 1,830 participants, performed a candidate gene analysis (30). The only positive association was with maternal homozygous SNP in FVL (Factor V Leiden) gene (2/488 [0.4%] vs. 1/1380 [0.0046%]; OR = 87.4; 95% confidence interval [95%CI]: 7.9–970.9). The investigators concluded that these heritable thrombophilia genetic markers were not associated with losses >20 weeks’ gestation. In another candidate gene study, pregnancy loss >22 weeks’ gestation was associated with carriers (n = 96) of allele A of rs1800783 eNOS (endothelial nitric oxide synthase 3) gene in placental tissue. The eNOS gene may be critical for pathways involved in placental growth (28). Furthermore, a genome-wide analysis using high-resolution Illumina SNP arrays identified 24 putative novel CNVs in placental and fetal samples (n = 54) (27). Using a larger study with similar methodology, Reddy et al. detected normal, abnormal (pathogenic), and variants of unknown significance CNVs in 396 (74.4%) samples from pregnancy loss >20 weeks’ gestation (including samples with anomalies) (3). The remainder of the studies examining losses >20 weeks’ gestation utilized other techniques such as quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), immunohistochemistry, and Western blot.

**Genetic Factors Associated With RPL**

Thirty-two studies (59%) examined RPL, including pre-embryonic, embryonic, and fetal losses, as the primary outcomes. There was variation in the definition of RPL across studies, with some using a minimum of two losses (34, 35) and others using a minimum of three (17, 36–42) (Table 3). The majority of RPL studies were hypothesis-based, i.e., conducted a candidate gene approach to examine SNPs in selected genes, a priori, and...
with plausible pathophysiologic pathways. Haplotype analysis conducted by Rogenhofer et al. showed that maternal blood M2 haplotype carriers with RPL \((n = 100)\) in \(ANXA5\), annexin 5 gene involved in coagulation, had a 3.4-fold increased RPL risk compared to controls \((n = 500)\) and a 2.1-fold increased RPL risk compared to randomly selected population controls \((n = 533)\) \((47)\). SNP-prevalence analysis conducted by Jin et al. showed RPL cases \((n = 112)\) carried the rs2249825 G allele in \(HMGB1\) (high mobility group box 1) gene in maternal whole blood more frequently than controls \((n = 118)\) \((48)\). Seyedhassani et al. compared the frequency of mutations in \(BAX\) gene, a pro-apoptotic gene, among RPL women \((n = 67)\) and controls \((n = 70)\) and showed associations between A(-179)G mutation in the \(BAX\) promoter and RPL \((41)\). Quintero-Ronderos et al. sequenced the complete coding region of \(THBD\), the endothelial cell receptor for thrombin gene, in women affected by RPL \((n = 262)\) and showed \(THBD\)-p.Trp153Gly mutation might be related to RPL \((54)\). Lastly, Masini et al. analyzed the genotype and allele frequencies of thrombin-activatable fibrinolysis inhibitor \(TAFI\) SNPs among women with \((n = 86)\) and without \((n = 72)\) RPL. Genotype and allele frequencies of \(TAFI\) +505 and +1583 SNPs were significantly different in women with RPL compared to controls \((38)\).

Genome-wide association studies of RPL were also conducted to highlight genetic variants with relevant functional pathways. For example, Kasak et al. examined placental and parental genome-wide CNV profiles of idiopathic RPL trios \((n = 53)\) parental blood, \(n = 13\) placental) and duos \((n = 8\) maternal blood, \(n = 9\) placental), and detected CNVs in \(NUP98\) and \(MTRR\) genes \((7)\). \(NUP98\) (Nucleoporin 98 And 96 Precursor) and \(MTRR\) (5-Methyltetrahydrofolate-Homocysteine Methyltransferase Reductase) genes are implicated in embryonic stem cell development and folate metabolism, respectively \((7)\). Another genome-wide association study was reported by Yu et al. \((31)\) but the study identified DNA methylation and gene expression, mechanisms that are also modulated by environmental factors \((31)\). The study suggested hypomethylation in \(CREB5\) gene in the decidual tissue was associated with RPL \((58)\).

Next generation sequencing approaches further identified deleterious mutations that are likely causal. For example, by conducting whole exome sequencing (WES) using parental...
TABLE 1 | Studies that reported genetic factors associated with EPL.

| PMID          | First author | Year | Pregnancy loss       | Predictor(s)                                      | Method                                      | Sample size | Tissue(s)                      | Reference |
|---------------|--------------|------|----------------------|--------------------------------------------------|---------------------------------------------|-------------|-------------------------------|-----------|
| 16738225      | Liu          | 2006 | Early Pregnancy Loss | Alteration of protein expression                  | Proteomic analysis                          | 12          | Placental chorionic villi     | (18)     |
| 23433743      | Ventura      | 2013 | Early Pregnancy Loss | Placental Expression of microRNA-17 and−19b       | Matched case-control expression microRNA analysis using qPCR | 31          | Placental chorionic villi     | (19)     |
| 24303885      | Cöl-Madendag | 2014 | Early Pregnancy Loss | Vascular endothelial growth factor (VEGF) expression | IHC                                          | 80          | Placental chorionic villi; endometrial decidua | (20)     |
| 26051097      | Fritz        | 2015 | Early Pregnancy Loss | Expression pattern of biomarker proteins in extravillous trophoblast (EVT) cells | Case-control study of trophoblast retrieval and isolation from the cervix from ongoing pregnancies | 20          | Endocervical specimens       | (21)     |
| 30074219      | Wu           | 2018 | Early Pregnancy Loss | TET family, 5-hmC expression                      | quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), western blotting and immunohistochemical (IHC) analyses | >3          | Placental chorionic villi     | (22)     |
| 29393376      | Hosseini     | 2018 | Early Pregnancy Loss | miRNAs (hsa-miRNA (miR)-125a-3p, hsa-miR-3663-3p, hsa-miR-423-5p and hsa-miR-575) | miRNA expression qRT-PCR analyses          | 24          | Maternal plasma; placental chorionic villi | (23)     |
| 29165645      | Hong         | 2018 | Early Pregnancy Loss | miR-378a-3p expression                            | qRT-PCR, western blotting, luciferase reporter assays | 105         | Endometrial decidua           | (24)     |
| 31203134      | He           | 2019 | Early Pregnancy Loss | Serum- and glucocorticoid-inducible kinase (SGK1) expression | Gene expression case-control analysis       | 67          | Placental chorionic villi     | (16)     |
| 19389728      | Sarno        | 2009 | Early Pregnancy Loss | HOX gene expression                               | qRT-PCR and western blotting analyses       | 46          | Endometrial decidua           | (25)     |

blood and placental chorionic villi samples, Qiao et al. (53) detected compound heterozygous deleterious mutations affecting DYNC2H1 and ALOX15 genes, both critical for early development, in two out of four families with RPL. Among unrelated women (n = 49) affected by RPL, Quintero-Ronderos et al. conducted WES in maternal leukocytes and detected 27 coding variants in 22 genes among 41% of the women. The affected genes, which were enriched by potentially deleterious sequence variants, belonged to distinct molecular cascades playing key roles in implantation (55). Furthermore, Shehab et al. conducted WGS analyses using maternal blood, unaffected offspring blood and fetal tissue in families (n = 7) with recurrent fetal death and detected a frameshift mutation in FOXP3 gene. The authors confirmed the mutation in the affected fetal tissue using Sanger sequencing.

Genetic Factors Associated With Unclassified Pregnancy Loss

Three studies were based on unclassified pregnancy loss, assessed over a broad range of gestational ages (Table 4). Cochery-Nouvellon et al. conducted a candidate gene study using 3,218 case (experienced embryonic loss at <10 weeks and fetal loss ≥10 weeks gestation) and 6,436 control mother-father pairs, the largest 1:2 matched case-control family-based study included in our review (66). The authors reported that the A6936G allele of PROCR, an endothelial protein C receptor gene involved in coagulation (Table 5), in maternal and paternal blood is associated with fetal death. The authors confirmed the association between candidate gene Factor V Leiden (F5), also involved in coagulation, and fetal loss, but pointed out that relationship between thrombophilias and pregnancy loss varies according to ethnicity and loss type. Alonso et al. (64) also examined mutations in the F5 gene in first-trimester abortions (at ≤12 weeks of gestation), second-trimester abortions (at 13–22 weeks of gestation), and fetal death (at ≥23 weeks) of mothers (n = 75). The presence of thrombophilia in 75% of the women combined with a mutation in F5 gene was marginally associated with intrauterine fetal death (P = 0.04; OR = 12; 95%CI: 1.44–102).

Genetic/Multi-Omic Pathways of Pregnancy Loss

Among the 54 studies included in this review, 26 (48%) examined placental tissue (e.g., chorionic villous tissue and
TABLE 2 | Studies that reported genetic factors associated with losses >20 weeks’ gestation.

| PMID       | First author | Year | Pregnancy loss | Predictor(s) | Method                                                                 | Sample size | Tissue(s)                  | Reference |
|------------|--------------|------|----------------|--------------|----------------------------------------------------------------------|-------------|----------------------------|-----------|
| 15963226   | Wicherek     | 2005 | Loss ≥24 weeks’ gestation | Placental RCAS1 expression | Western blot method with the use of monoclonal anti-RCAS1 antibody     | 67          | Placental                  | (28)      |
| 21732394   | Harris       | 2011 | Loss ≥22 weeks’ gestation | Genomic structural variations; CNVs | Genome-wide analysis using high-resolution Illumina SNP arrays (Human CNV370-Duo) | 54          | Placental tissue; fetal tissue | (27)      |
| 23021696   | Ferrari      | 2012 | Loss ≥22 weeks’ gestation | SNPs in endothelial nitric oxide synthase (eNOS) gene | Case-control candidate SNP association                                 | 96          | Placental tissue           | (28)      |
| 23215556   | Reddy        | 2012 | Loss ≥20 weeks’ gestation | CNVs of at least 500 kb | Chromosomal microarray analysis (case-only)                             | 532         | Placental tissue; fetal tissue | (3)       |
| 26094028   | Ernst        | 2015 | Loss ≥23 weeks’ gestation | Fetal copy-number variation (CNV) | Retrospective case-control microarray and qPCR analyses                | 94          | Umbilical cord             | (29)      |
| 27131585   | Silver       | 2016 | Loss ≥20 weeks’ gestation | Maternal factor V Leiden; fetal PAI-1 4G/4G polymorphism | Case-control candidate single nucleotide polymorphism (SNP) association | 1,830       | Maternal serum; fetal cord blood; placental chorionic villi | (30)     |
| 26827687   | Romagnuolo   | 2016 | Loss ≥24 weeks’ gestation | Lp(a) levels measurement | Retrospective observational study                                     | 630         | Maternal blood leukocytes; maternal blood | (31)      |
| 26004986   | Ferrari      | 2016 | Loss ≥22 weeks’ gestation | Placental telomere shortening | qPCR of 42 unexplained stillbirths (>22 weeks), 43 term and 15 preterm live births | 100         | Placental tissue           |           |
| 28645573   | Maiti        | 2017 | Loss ≥32 weeks’ gestation | Aldehyde oxidase 1 and G-protein-coupled estrogen receptor 1 | IHC and gene expression analyses using qRT-PCR                         | 4           | Placental chorionic villi  | (32)      |
| 28990860   | Campbell     | 2018 | Loss ≥24 weeks’ gestation | Genetic test results, placental pathology | Review of pathology reports and collected demographic data on cases | 131         | Placental                  | (33)      |

In this review, we identified 54 research studies that reported genetic/multi-omic etiologies underlying pregnancy loss. Two studies (4%) incorporated samples from parent-offspring trios (maternal, paternal and fetal/placental) and identified genetic factors related to recurrent losses. Twenty-three studies (53%) examined genetic factors assessed in the maternal tissue samples only (Figure 2). Multiple genetic pathways associated with embryonic and fetal survival may play a role in pregnancy loss. The reported pathways are essential for placental function, epigenetic reprogramming, embryonic development and several critical cellular functions (Table 5).

DISCUSSION

In this review, we identified 54 research studies that reported genetic/multi-omic etiologies underlying pregnancy loss. Twenty-six studies examined DNA from placental and/or fetal tissues, including two studies with maternal and paternal samples, and supported their findings on genetic abnormalities associated with pregnancy loss. Based on data from studies included in this review, multiple genes with functional pathways that may be essential for embryonic/fetal survival were discussed.

Genetic Factors Associated With Pregnancy Loss

Eight studies reported genetic/multi-omic etiologies of EPL, however, the studies examined miRNAs, including other epigenetic regulators and proteins that require utilization of expensive targeted assays (e.g., qRT-PCR and immunohistochemistry). Epigenetic mechanisms may play an important role in placental development and function, but are also modulated by environmental factors (7). Indeed, the etiology of many pregnancy losses could be multifactorial, including genetic and environmental factors; however, in some couples, pregnancy loss can be inherited as a Mendelian trait (i.e., monogenic form) (67). Despite the strong genetic underpinnings underlying EPL (10, 68), evidence for causal genetic variants is lacking.
| PMID       | First author | Year | Pregnancy Loss | Predictor(s)                                                                 | Method                                                                 | Sample size | Tissue(s)                  | Reference |
|------------|--------------|------|----------------|------------------------------------------------------------------------------|----------------------------------------------------------------------|-------------|---------------------------|-----------|
| 31396989   | Zhang        | 2019 | Recurrent Pregnancy Loss | NOD1 gene expression                                                        | Gene expression case-control analysis                                | 38          | Endometrial decidua       | (43)      |
| 17099210   | Kaare        | 2007 | Recurrent Pregnancy Loss | Variations in the thrombomodulin and endothelial protein C receptor genes     | Case-control family (couples) mutation detection using liquid chromatography | 277         | Maternal blood; paternal blood | (44)      |
| 21160146   | Ticconi      | 2009 | Recurrent Pregnancy Loss | Genotype allele frequency of Beta-Fibrinogen G-455A                          | Case-control study                                                    | 176         | Maternal blood            | (45)      |
| 11857060   | Wang         | 2002 | Recurrent Pregnancy Loss | Polymorphism of the IL-1beta gene (IL1B)                                    | Retrospective case-control study SNP frequency                        | 59          | Peripheral blood; mononuclear cells (PBMCs) derived from trophoblast cell line | (38)      |
| 12874795   | Choi         | 2003 | Recurrent Pregnancy Loss | Expression of angiogenesis and apoptosis related genes                       | qRT-PCR analysis                                                      | 12          | Placental chorionic villi | (42)      |
| 16253969   | Wang         | 2006 | Recurrent Pregnancy Loss | Maternal CD46H2 and IL1B-5111 Homozygosity in T Helper 1-type Immunity to Trophoblast Antigens | Case-control study                                                    | 203         | Trophoblast tissue        | (37)      |
| 18774564   | Masini       | 2009 | Recurrent Pregnancy Loss | Thrombin-activatable fibrinolysis inhibitor (TAFI) single nucleotide polymorphisms (SNPs) | Case-control study                                                    | 158         | Maternal blood            | (38)      |
| 21996032   | Park         | 2011 | Recurrent Pregnancy Loss | Kisspeptin expression                                                        | IHC, flow cytometry and gene expression analyses                      | 52          | Endometrial decidua; trophoblast tissue; maternal blood | (34)      |
| 20977975   | Elter        | 2011 | Recurrent Pregnancy Loss | Vascular Endothelial Growth factor-A Gene Polymorphisms                      | Case-control study allele frequency analysis                          | 280         | Placental tissue          | (39)      |
| 20962020   | Uusküla      | 2011 | Recurrent Pregnancy Loss | Methylthion Allic Polymorphism (MAP) in Chorionic Gonadotropin beta5 (CGFS)  | Methylation analysis                                                  | 32          | Trophoblast tissue        | (40)      |
| 22291743   | Seyyedhassani| 2011 | Recurrent Pregnancy Loss | Alterations of the Bax gene (a pro-apoptotic gene)                          | Case-control frequency of mutation detection using PCR                | 137         | Maternal blood            | (41)      |
| 22350224   | Saunders     | 2012 | Recurrent Pregnancy Loss | IgG(3) reactivity                                                            | Case and matched control comparison using Immunoprecipitation and Western immunoblotting analyses | 28          | Maternal serum            | (35)      |
| 22505054   | Kreig        | 2012 | Recurrent Pregnancy Loss | Gene expression alterations                                                  | Case-control microarray; gene expression; pathway, gene ontology (GO) and qRT-PCR analyses, qPCR and western blot analyses to examine differential expression between cases and controls | 16          | Endometrial decidua       | (17)      |
| 23850136   | Nair         | 2013 | Recurrent Pregnancy Loss | Inflammatory Proteins S100A8 and S100A9                                      | qPCR and western blot analyses to examine differential expression between cases and controls | 65          | Endometrial decidua       | (48)      |
### TABLE 3 | Continued

| PMID       | First author     | Year | Pregnancy loss          | Predictor(s)                                                                 | Method                                                                 | Sample size | Tissue(s)                  | Reference |
|------------|------------------|------|-------------------------|------------------------------------------------------------------------------|-------------------------------------------------------------------------|-------------|-----------------------------|-----------|
| 23498654   | Rogenhofer       | 2013 | Recurrent Pregnancy Loss | M2 haplotype of ANXA5 gene                                                   | Comparing M2/ANXA5 genotype among 100 PCOS, 500 fertile and 533 random population control women | 1,133       | Maternal blood              | (47)      |
| 25956284   | Jin              | 2015 | Recurrent Pregnancy Loss | HMGB1 rs2249825C/G and rs1412125T/C polymorphisms                            | Case-control study of PCR-restriction fragment length polymorphism assay analyses | 230         | Placental chorionic villi   | (48)      |
| 25925347   | Perfetto         | 2015 | Recurrent Pregnancy Loss | IL-22 levels                                                                 | qPCR, Western blot, and IHC                                              | 20          | Endometrial decidua         | (49)      |
| 27535548   | He               | 2016 | Recurrent Pregnancy Loss | Early pregnancy Cx43 and VEGF mRNA and protein expression                     | IHC, western blot, and qRT-PCR analyses                                 | 56          | Placental chorionic villi; endometrial decidua | (50)      |
| 27477959   | Yan              | 2016 | Recurrent Pregnancy Loss | 1st trimester Vitamin D receptor (VDR) expression                            | Evaluation by IHC, confocal laser scanning microscopy (CLSM), western blot, qPCR, and enzyme-linked immunosorbent assay analyses | 80          | Placental chorionic villi; endometrial decidua | (51)      |
| 27929073   | Sober            | 2016 | Recurrent Pregnancy Loss | Gene expression alterations                                                   | Case-control RNA differential sequencing (DESeq) analysis               | 10          | Placental chorionic villi   | (52)      |
| 26826184   | Qiao             | 2016 | Recurrent Pregnancy Loss | DNA alterations within exons                                                  | Case-only family WES                                                    | 4           | Maternal blood; paternal blood; placental chorionic villi | (53)      |
| 28345811   | Kasak            | 2017 | Recurrent Pregnancy Loss | NUP98 (embryonic stem cell development) and MTRR (folate metabolism) genes | Copy number variant (CNV) analysis of idiopathic RPL trios (mother-father-placenta) and duos (mother-placenta) | 79          | Maternal blood; paternal blood; placental chorionic villi | (7)       |
| 29195508   | Quintero-Ronderos| 2017 | Recurrent Pregnancy Loss | Endothelial cell receptor for thrombin gene (THBD)                           | Case-control coding sequence mutation detection using bioinformatics    | 262         | Maternal blood              | (54)      |
| 29016666   | Quintero-Ronderos| 2017 | Recurrent Pregnancy Loss | DNA alterations within exons                                                  | Case-only whole exome sequencing (WES)                                  | 49          | Maternal blood leukocytes   | (55)      |
| 28833278   | Shehab           | 2017 | Recurrent Pregnancy Loss | FOXP3 gene frameshift mutations (p.D303fs*87)                                 | Whole genome sequencing of families                                     | 7           | Maternal blood; unaffected offspring blood; fetal tissue | (56)      |
| 30349621   | Li               | 2018 | Recurrent Pregnancy Loss | ITI-H4 and plasma kallikrein (KLKB1)                                       | Gene expression case-control analysis                                   | 90          | Maternal serum; maternal blood | (57)      |
| 30100398   | Yu               | 2018 | Recurrent Pregnancy Loss | CREB5 expression                                                             | Genome-wide DNA methylation and gene expression analyses               | 100         | Endometrial decidua         | (58)      |
| 24557735   | Papamitsou       | 2014 | Recurrent Pregnancy Loss | Expressions of HLAG (Human Leukocyte Antigen G), CD68 (Cluster of Differentiation 68), CD56, CD16 and CD25 during pregnancy | IHC                                                                     | 50          | Endometrial decidua         | (59)      |
| 11279300   | Pfeiffer         | 2001 | Recurrent Pregnancy Loss | Human leukocyte antigen (HLA)-G genotype                                      | Case-control comparison of haplotypes                                   | 130         | Maternal blood; paternal blood | (60)      |

(Continued)
TABLE 3 | Continued

| PMID | First author | Year | Pregnancy loss | Predictor(s) | Method | Sample size | Tissue(s) | Reference |
|------|--------------|------|----------------|--------------|--------|-------------|----------|-----------|
| 16403802 | Kaare | 2006 | Recurrent Pregnancy Loss | Homozygous mutations in the Amnionless (AMN) gene | Case-only Families (couples) sequence variation detection using liquid chromatography | 85 | Maternal blood; paternal blood | (61) |
| 25457193 | Agrawal | 2015 | Recurrent Pregnancy Loss | HLA-G S’ upstream regulatory region SNPs | Case-control comparison of haplotypes | 200 | Maternal blood; paternal blood | (62) |
| 24621454 | Gharesi-Fard | 2014 | Recurrent Pregnancy Loss | Proteins involved in proliferation and migration of endothelial cells as well as control of coagulation | Differential expression analysis using qPCR and Western blot techniques | 10 | Placental tissue | (63) |

TABLE 4 | Studies that reported genetic factors associated with unclassified pregnancy loss.

| PMID | First author | Year | Pregnancy loss | Predictor(s) | Method | Sample size | Tissue(s) | Reference |
|------|--------------|------|----------------|--------------|--------|-------------|----------|-----------|
| 12439528 | Alonso | 2002 | Unclassified Pregnancy Loss | Mutations of factor V Leiden, methylenetetrahydrofolate reductase, and prothrombin gene | Case-control ELISA analysis | 150 | Maternal blood | (64) |
| 30136429 | Mehndjiev | 2018 | Unclassified Pregnancy Loss | MTHFR C677T TT genotype and T allele | Cross-sectional study | 243 | Endometrial decidua | (65) |
| 19806250 | Cochery-Nouvellon | 2009 | Unclassified Pregnancy Loss | A6936G allele of the endothelial protein C receptor (EPCR) gene (PROCR) | 1:2 case-control study | 9,654 | Maternal blood; paternal blood | (66) |

Among genome-wide association studies of pregnancy loss at 20 weeks’ gestation or more, two studies utilized chromosomal microarray, a higher resolution and enhanced sensitivity method that allowed unbiased detection of pathogenic abnormalities (3, 27). These studies by Reddy et al and Harris et al detected 24 putative novel CNVs in 54 placental and fetal samples from losses >20 and 22 weeks’ gestation, respectively, and genetic abnormalities explained 41.9% of idiopathic cases (3, 27). A recent study, that was not included in our review due to its publication date, improved these findings by utilizing NGS approach that allowed detection of the de novo lethal mutations and the “intolerance” (i.e., genes that are critical for human development, the loss of which is incompatible with life) (11). Using the maternal and fetal samples, enrichment of loss-of-function variants in genes that are intolerant to variation in the human population were observed. This suggested dramatic and progressive increases in the proportion of losses >20 weeks’ gestation with likely causative genetic abnormalities, however, the genetic etiologies of 40% of idiopathic cases remain to be elucidated. Due to unavailability of paternal samples in the previous studies, they could not detect compound heterozygous deletions, distinguish pathogenic de novo from inherited variants and consequently could not explain significant proportion of idiopathic cases. Additional efforts were made by Cochery-Nouvellon et al. (66) that utilized mother-father duos with larger sample size. However, the study was a candidate gene study and showed limited evidence of association between coagulation pathway genes and unclassified pregnancy loss.

Among thirty-two studies that reported genetic etiologies of RPL, making up the majority of studies included in this review, two utilized an NGS approach in families to identify deleterious mutations that are likely causal (53, 56). Using WES analysis in parental blood and placental chorionic villi samples, Qiao et al. (53) detected compound heterozygous deleterious mutations affecting DYNC2H1 and ALOX15, genes critical for early development, in two out of four families with RPL. By conducting WGS followed by Sanger sequencing validation analyses, Shehab et al. (56) detected frameshift mutation in FOXP3 gene that is critical for the function of regulatory T cells in families affected by recurrent intrauterine fetal death. Other genes such as loss-of-function risk variants and inherited pathogenic mutations in intolerant genes were not identified, potentially due to the lack of larger parent–offspring trio studies.

Guide to Next Steps in Determining Genetic Factors Associated With Pregnancy Loss

While chromosomal microarray, the current clinical guideline for genetic evaluation of losses >20 weeks’ gestation, enhanced
Table 5: Reported genetic/multi-omic pathways in relation to gestational age specific pregnancy.

| Pregnancy loss phenotype | Genes, microRNAs, mRNAs, or chromosomes | Functional pathway | Number of studies |
|--------------------------|------------------------------------------|-------------------|------------------|
| Early pregnancy loss     | SGK1, miR-575, miRNA-17, miRNA-19b, VEGF | Placental function | 7                |
|                          | TET family, 6-hmC                         | Epigenetic reprogramming |              |
|                          | miR-125a, miR-3663-3p                     | Mitosis, meiosis, cell cycle progression |        |
|                          | miR-3663-3p, miR-135a, miR-122, let-7, miR-378a-3p | Apoptosis |              |
|                          | miR-125a                                  | Hematopoiesis |              |
|                          | miR-125a, miR-135a                        | Implantation |              |
|                          | HOX family                                | Endometrial function |           |
| Losses >20 weeks’ gestation | F5, PAI-1, eNOS                          | Coagulation | 6               |
|                          | AOX-1, GPER                               | Oxidation and cellular aging |           |
|                          | LPA                                       | Lipoprotein synthesis |           |
|                          | Ch 1p13.3, NOS3, ROAS1                    | Inflammation and immunity |           |
|                          | eNOS                                      | Mitosis, cell cycle progression |           |
|                          | eNOS                                      | Vascular tone |           |
| Recurrent pregnancy loss | NOD1, F11-H4, KLB1, IL-22, HLAG, CD16, CD66, CD56, S100A8, S100A9, KISS1, IL1B, CO46, FOXP3, NLRP2, NLRPS, NLRP7, IDO2 | Inflammation and immunity | 32               |
|                          | CREB5, DYNDC2H1, PLC4D4, OSBPL5, STIL     | Mitosis, meiosis, cell cycle progression |          |
|                          | CREB5, BAX, CASP9                         | Apoptosis |          |
|                          | NUP98, IFT122, APAF1, CASP9, CSP1, NLRP5, PAD6 | Embryonic development |          |
|                          | MTRR, VDR                                | Folate and other vitamin metabolism |           |
|                          | Cx43, VEGF, ALOX15                        | Placental function |           |
|                          | Cx43, VEGF, VEGF, VEGFA, FL1, EPAS1       | Angiogenesis |           |
|                          | ANXA5, TAF, THBD, FGA, FGB, PROCR         | Coagulation |           |
|                          | KISS1, CHRNA1, RYR1, MUSK                 | Cell signaling |           |
|                          | CGB5                                      | Implantation |           |
|                          | KIF14, IFT122, DYNDC2H1                   | Ciliogenesis |           |
|                          | MMP10                                     | Extracellular matrix organization |           |
|                          | CAPS                                      | Ion transport |           |
| Unclassified fetal death | PROC, F5, F2                             | Coagulation | 3               |
|                          | MTHFR                                     | Folate and other vitamin metabolism |           |

The ability to detect microdeletions and duplications beyond the resolution of standard karyotype, additional detailed diagnostic yields will require utilization of NGS approach. Efforts are underway to apply this technology to losses >20 weeks’ gestation (69).

With the advent of NGS, monogenic disorders (including de novo, inherited autosomal-dominant/autosomal-recessive mutations, and SNPs) that are either lethal, known to cause disease, or dramatically increase risk of pregnancy loss in families can be identified. De novo mutations occur as likely penetrant variation in a Mendelian gene and could explain sporadic cases of pregnancy loss. Point mutations, other genetic variations such as CNVs (genomic deletions or duplications), as identified by studies in this review, may also occur de novo. The added contribution of novel de novo missense variants to losses >20 weeks’ gestation was estimated by pulling all rare and damaging novel missense variants in the study (111). Therefore, the authors estimated a bound on the diagnostic yield in known genes associated with losses >20 weeks’ gestation between the previously reported yield (4.5%) vs. the present yield (13.4%; 36/268 cases). However, without parental genotype information, the study remained at the lower bound of the diagnostic yield. Consistent with other diagnostic studies, the diagnostic yield using parent-offspring trios is estimated to be up to three-fold higher compared with studies that use singletons (70).
Combined with identification of de novo mutations, other single gene abnormalities may be used to provide prognosis based on data from other patients with similar mutations (71). Such monogenic forms may be associated with extreme phenotypes and early losses, but this is not always the case. Studies that show familial aggregation of pregnancy loss may help clarify whether losses that occur early in gestation and a positive family history exists, suggesting autosomal-dominant transmission of risk alleles. To prove whether the mutations appeared in the germline of the probands as de novo mutations, parental DNA assessment is required (67).

Challenges still remain in clinical applications of genome sequencing and validating the results from sequencing using maternal cell-free DNA, chorionic villus sampling and amniocentesis. Suggested strategies to overcome these challenges include serial assessment of genotypes, phenotypes and ‘omics data over the course of the pregnancy (e.g., genomics, transcriptomics, metabolomics) (10, 68). Molecular diagnostic evaluations rely on databases (e.g., OMIM) and guidelines of the American College of Medical Genetics and Genomics with characteristics designed to enrich for pathogenicity in Mendelian disease genes (11). In these databases, lethal phenotypes are especially poorly represented. Other strategies for gene discovery, including determination of the “intolerome” are likely to reveal new genotype-phenotype correlations and shed light on the human “intolerome,” conditions incompatible with life resulting in fetal demise (11, 13). Studies that incorporate DNA sequencing in affected and unaffected families, designed as case-control trio studies, will help in determination of the “intolerome” by identifying novel embryonic-lethal or fetal-lethal variants that are not seen in unaffected families. Using WGS in parent-offspring trios, 60–80 high confidence de novo mutations per individual can be identified (67). Compared with WES, WGS may further expand the spectrum of causal de novo mutations by allowing for a better coverage of the exome and identification of non-coding variants.

Limitations and Strengths of the Systematic Review

Although PubMed search is a comprehensive retrieval tool appropriate for systematic review of journal research in health care, other search methods (e.g., Embase, Web of Science) were not utilized. Restricted MeSH terms applied in PubMed may have excluded other studies pertinent to the present systematic review. To provide a more comprehensive review of the literature, we reviewed and included studies within review articles that matched eligibility in our search criteria. In addition, we independently explored OMIM to report and confirm genetic pathways and functional effects of the reported genes.

Guide to Next Steps

Experts have recommended categorization of pregnancy loss as: < 10 weeks gestational age (termed early pregnancy loss), 10–19 weeks and 6 days of gestation (termed fetal death), and 20 or more weeks gestation (termed stillbirth). EPL was further subdivided into peri-implantational loss before 5 weeks, pre-embryonic loss from 5 to 5 weeks and 6 days of gestation, and embryonic loss from 6 to 9 weeks and 6 days of gestation (14). Similarly, fetal death can be subdivided into early fetal death, defined as losses between 10 and 15 weeks and 6 days of gestation, and late fetal death, losses from 16 to 19 weeks and 6 days of gestation (14). These classifications may identify studies that report genetic factors with different mechanisms, e.g., genes essential for embryonic lethality and functional genes essential for human development (e.g., cardiomyopathy). Additionally, assessment of losses at different stages of pregnancy may help identify pathways essential for in utero survival at critical stages of development.

CONCLUSION

Pregnancy loss is multi-factorial, but recent studies identified genetic pathways essential for embryonic and fetal survival. Further research systematically evaluating pregnancy loss across various developmental epochs and utilizing NGS in families may identify single-gene mutations causing embryonic/fetal loss and that are not found in healthy controls. Identification of such genes and their pathways may provide novel biomarkers for risk stratification and therapeutic targets to improve pregnancy outcomes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article-supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

The literature search was conducted and cross-examined by AC and TW. AC, NB, MV, TW, and RS directed its implementation. AC and TW drafted the manuscript. All authors reviewed the article and revised it critically for important intellectual content, and all authors provided final approval of the draft being submitted.
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REFERENCES

1. Fedor Nilsson S, Andersen PK, Strandberg-Larsen K, Nybo Andersen A-M. Risk factors for miscarriage from a prevention perspective: a nationwide follow-up study. BJOG: Int J Obstetrics Gynaecol. (2014) 121:94. doi: 10.1111/1471-0528.12694
2. Rossen LM, Ahrens KA, Branum AM. Trends in risk of pregnancy loss among us women, 1990–2011. Paediatric Perinatal Epidemiol. (2018) 32:19–29. doi:10.1111/ppe.12417
3. Reddy UM, Page GP, Saade GR, Silver RM, Thorsten VR, Parker CB, et al. Karyotype versus microarray testing for genetic abnormalities after stillbirth. N Eng J Med. (2012) 367:2185–93. doi: 10.1056/NEJMoa1201569
4. Frias AE, Luikenaar RA, Sullivan AE, Lee RM, Porter TF, Branch DW, et al. Poor obstetric outcome in subsequent pregnancies in women with prior fetal death. Obstet Gynecol. (2004) 104:521–6. doi: 10.1097/00006255-200410000-00015
5. No RGG. The Investigation and Treatment of Couples With Recurrent First-trimester and Second-trimester Miscarriage. RCOG: London (2011).
6. Warren JE, Silver RM. Genetics of pregnancy loss. Clin Obstetric Gynecol. (2008) 51:84–95. doi: 10.1097/GE.0b013e318161719c
7. Kasak L, Rull K, Sõber S, Laan M. Copy number variation profile in the placental and parental genomes of recurrent pregnancy loss families. Sci Rep. (2017) 7:45327. doi: 10.1038/srep45327
8. Branch DW, Heuser C. “Recurrent Miscarriage,” In: Carrell CM, Petersen DT, editors. Reproductive Endocrinology Infertility: Integrating Modern Clinical Laboratory Practice (New York, NY: Springer), 281–296.
9. Wilson RD, Gagnon A, Audibert F, Campagnolo C, Carroll J, Wilson RD, et al. Poor obstetric outcome in subsequent pregnancies in women with prior fetal death. Obstet Gynecol. (2004) 104:521–6. doi: 10.1097/00006255-200410000-00015
10. Blue NR, Page JM, Silver RM. Genetic abnormalities and pregnancy loss. Semin Perinatol. (2019) 43:66–73. doi: 10.1053/j.semperi.2018.12.002
11. Stanley KE, Giordano J, Thorsten V, Buchovecky C, Thomas A, Ganapathi M, et al. Causal genetic variants in stillbirth. Mol Med Rep. (2019) 118:1402–8. doi: 10.1177/1991612219825730
12. Pereza N, Ostojic S, Kapovic M, Peterlin B. Systematic review and meta-analysis of genetic association studies in idiopathic recurrent spontaneous abortion. Fertil Steril. (2017) 107:150–9. doi: 10.1016/j.fertnstert.2016.10.007
13. Gray KJ, Wilkins-Haug L. Special issue on “Feto-Maternal Genomic part by the National Center for Advancing Translational Research reported in this publication was supported in
14. Kasak L, Rull K, Sõber S, Laan M. Copy number variation profile in the
15. Kasak L, Rull K, Sõber S, Laan M. Copy number variation profile in the placental and parental genomes of recurrent pregnancy loss families. Sci Rep. (2017) 7:45327. doi: 10.1038/srep45327
16. Branch DW, Heuser C. “Recurrent Miscarriage,” In: Carrell CM, Petersen DT, editors. Reproductive Endocrinology Infertility: Integrating Modern Clinical Laboratory Practice (New York, NY: Springer), 281–296.
17. Wilson RD, Gagnon A, Audibert F, Campagnolo C, Carroll J, Wilson RD, et al. Poor obstetric outcome in subsequent pregnancies in women with prior fetal death. Obstet Gynecol. (2004) 104:521–6. doi: 10.1097/00006255-200410000-00015
18. Blue NR, Page JM, Silver RM. Genetic abnormalities and pregnancy loss. Semin Perinatol. (2019) 43:66–73. doi: 10.1053/j.semperi.2018.12.002
19. Stanley KE, Giordano J, Thorsten V, Buchovecky C, Thomas A, Ganapathi M, et al. Causal genetic variants in stillbirth. Mol Med Rep. (2019) 118:1402–8. doi: 10.1177/1991612219825730
20. Pereza N, Ostojic S, Kapovic M, Peterlin B. Systematic review and meta-analysis of genetic association studies in idiopathic recurrent spontaneous abortion. Fertil Steril. (2017) 107:150–9. doi: 10.1016/j.fertnstert.2016.10.007
21. Gray KJ, Wilkins-Haug L. Special issue on “Feto-Maternal Genomic part by the National Center for Advancing Translational Research reported in this publication was supported in
14. Kasak L, Rull K, Sõber S, Laan M. Copy number variation profile in the placental and parental genomes of recurrent pregnancy loss families. Sci Rep. (2017) 7:45327. doi: 10.1038/srep45327
16. Branch DW, Heuser C. “Recurrent Miscarriage,” In: Carrell CM, Petersen DT, editors. Reproductive Endocrinology Infertility: Integrating Modern Clinical Laboratory Practice (New York, NY: Springer), 281–296.
17. Wilson RD, Gagnon A, Audibert F, Campagnolo C, Carroll J, Wilson RD, et al. Poor obstetric outcome in subsequent pregnancies in women with prior fetal death. Obstet Gynecol. (2004) 104:521–6. doi: 10.1097/00006255-200410000-00015
18. Blue NR, Page JM, Silver RM. Genetic abnormalities and pregnancy loss. Semin Perinatol. (2019) 43:66–73. doi: 10.1053/j.semperi.2018.12.002
19. Stanley KE, Giordano J, Thorsten V, Buchovecky C, Thomas A, Ganapathi M, et al. Causal genetic variants in stillbirth. Mol Med Rep. (2019) 118:1402–8. doi: 10.1177/1991612219825730
20. Pereza N, Ostojic S, Kapovic M, Peterlin B. Systematic review and meta-analysis of genetic association studies in idiopathic recurrent spontaneous abortion. Fertil Steril. (2017) 107:150–9. doi: 10.1016/j.fertnstert.2016.10.007
21. Gray KJ, Wilkins-Haug L. Special issue on “Feto-Maternal Genomic part by the National Center for Advancing Translational Research reported in this publication was supported in
14. Kasak L, Rull K, Sõber S, Laan M. Copy number variation profile in the placental and parental genomes of recurrent pregnancy loss families. Sci Rep. (2017) 7:45327. doi: 10.1038/srep45327
16. Branch DW, Heuser C. “Recurrent Miscarriage,” In: Carrell CM, Petersen DT, editors. Reproductive Endocrinology Infertility: Integrating Modern Clinical Laboratory Practice (New York, NY: Springer), 281–296.
17. Wilson RD, Gagnon A, Audibert F, Campagnolo C, Carroll J, Wilson RD, et al. Poor obstetric outcome in subsequent pregnancies in women with prior fetal death. Obstet Gynecol. (2004) 104:521–6. doi: 10.1097/00006255-200410000-00015
18. Blue NR, Page JM, Silver RM. Genetic abnormalities and pregnancy loss. Semin Perinatol. (2019) 43:66–73. doi: 10.1053/j.semperi.2018.12.002
19. Stanley KE, Giordano J, Thorsten V, Buchovecky C, Thomas A, Ganapathi M, et al. Causal genetic variants in stillbirth. Mol Med Rep. (2019) 118:1402–8. doi: 10.1177/1991612219825730
34. Park D-W, Lee S-K, Hong SR, Han A-R, Kwak-Kim J, Yang KM. Expression of KISSpeptin and its receptor GPR54 in the first trimester trophoblast of women with recurrent pregnancy loss. Am J Reprod Immunol. (2010) 63:75–92. doi: 10.1111/j.1600-0897.2010.01073.x

35. Saunders RD, Nakajima ST, Rai SN, Pan J, Gercel-Taylor C, Taylor DD. Alterations in antibody subclass immune reactivity to trophoblast-derived fetal fibronectin and α2-macroglobulin in women with recurrent pregnancy loss. Am J Reprod Immunol. (2012) 68:438–49. doi: 10.1111/1600-0897.2011.01182.x

36. Wang ZC, Yunis EJ, De los Santos MJ, Xiao L, Anderson DJ. Maternal CD46H∗. Mol Reprod Dev. (2006) 71:818–22. doi: 10.1002/mrd.20168

37. Masini S, Ticconi G, Gravina P, Tomassini M, Pietropolli A, Forte V, et al. Thrombin-activatable fibrinolysis inhibitor polymorphisms and recurrent pregnancy loss. Fertil Steril. (2009) 92:694–702. doi: 10.1016/j.fertnstert.2008.07.015

38. Choi H-K, Choi BC, Lee S-H, Kim JW, Cha KY, Baek K-H. Expression of interleukin-22 in decidua of patients with early pregnancy loss. J Reprod Immunol. (2011) 87:1–7. doi: 10.1016/j.jri.2010.06.159

39. Nair RR, Khanna A, Singh K. Role of inflammatory proteins S100A8 and S100A9 in pathophysiology of recurrent early pregnancy loss. Placenta. (2013) 34:824–7. doi: 10.1016/j.placenta.2013.06.037

40. Gothenhofer N, Engels L, Bogdanova N, Tüttelmann F, Thaler CJ, Markoff A. Independent association of the M2/A2AX5 haplotype with recurrent pregnancy loss (RPL) in PCOS patients. Metab Clin Exp. (2013) 62:1057–60. doi: 10.1016/j.metabol.2013.02.005

41. He X, Chen Q. Reduced expressions of connexin 43 and VEGF in the first-trimester tissue from women with recurrent pregnancy loss. Reprod Biol Endocrinol. (2016) 14:66. doi: 10.1186/s12958-016-0179-4

42. Yan X, Wang L, Yan C, Zhang X, Hui L, Sheng Q, et al. Decreased expression of the vitamin D receptor in women with recurrent pregnancy loss. Arch Biochem Biophys. (2016) 606:128–33. doi: 10.1016/j.abb.2016.07.021

43. Söber S, Rull K, Reiman M, Illsson P, Mattila P, Laan M. RNA sequencing of villi from recurrent pregnancy loss patients reveals impaired function of basic nuclear and cellular machinery. Sci Rep. (2016) 6:38439. doi: 10.1038/srep38439

44. Quintero-Ronderos P, Mercier E, Gris J-C, Esteban-Perez C, Moreno-Ortiz H, Fonseca DJ, et al. THBD sequence variants potentially related to recurrent pregnancy loss. Reprod Biol Endocrinol. (2017) 15:92. doi: 10.1186/s12958-017-0311-0

45. Carey et al. Genetics of Pregnancy Loss
71. Acuna-Hidalgo R, Veltman JA, Hoischen A. New insights into the generation and role of de novo mutations in health and disease. *Genome Biol.* (2016) 17:1–19. doi: 10.1186/s13059-016-1110-1

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