American Journal of Biomedical Science & Research

www.biomedgrid.com

ISSN: 2642-1747

Mini Review

Genetic Studies of Pregnancy Loss

Xinliang Zhao[1] and Nanbert Zhong[2]*

[1]*Peking University Center of Medical Genetics, China*

[2]*New York State Institute for Basic Research in Developmental Disabilities, USA*

***Corresponding author:** Nanbert Zhong, MD, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314, USA

To Cite This Article: *Xinliang Zhao, Nanbert Zhong. Genetic Studies of Pregnancy Loss. Am J Biomed Sci & Res. 2021 - 12(5). AJBSR.MS.ID.001782.* DOI: 10.34297/AJBSR.2021.12.001782.

Received: April 13, 2021; **Published:** April 27, 2021

Abstract

Pregnancy loss is known as the death of an unborn fetus at any time during pregnancy. It is a complex disorder that affects 10% of clinical pregnancies. Both genetic and environmental factors are associated with the condition. Various experimental techniques, including conventional karyotyping, array-based applications, and whole exon/genome sequencing, have contributed to discovering the genetic pathology of pregnancy loss. Studies have shown that abnormalities such as aneuploidy, translocation, copy number variation, mutation, mosaicism, and epigenetic changes are involved in the development of pregnancy loss. Here, we review previous studies that utilized cytogenetic and molecular genetic tools to investigate the cause of pregnancy loss in terms of the whole genome.

Keywords: Pregnancy loss; Miscarriage; Stillbirth; Genetics

Introduction

Pregnancy loss (PL) is widely recognized as the death of an unborn fetus at any time during pregnancy [1]. According to official data, PL occurs in approximately 30% of conceptions and 10% of clinically acknowledged pregnancies [2]. The causes of the loss vary, depending on the timing of the loss, with an increased likelihood of a genetic cause in the early stage of pregnancy. PL is categorized according to its period of gestation: preimplantation, pre-embryonic (post-implantation, but embryo not visible under ultrasonography), embryonic (embryo visible under ultrasonography, and gestational weeks [GW] < 10), early fetal (10–13 GW), late fetal (14–19 GW), or stillbirth (≥ 20 GW) [3].

The genetic contribution to PL was first investigated in 1975 [4]. The single leading cause of PL at the embryonic stage or even earlier is harboring a major chromosomal abnormality (mainly aneuploidies) in the conceptus, which is responsible for more than 50% of loss occurring in the 1st trimester [5]. While for stillbirth, the contribution of chromosomal abnormalities drops to approximately 8–10%. Recurrent pregnancy loss (RPL) is a more devastating multifactorial problem. It has been defined as two or more failed pregnancies [6,7]. Genetic disturbance, whether occurring in the conceptus or the parents, both can predispose to sporadic pregnancy loss (SPL) or RPL.

There are more enthusiasm and justification of genetic testing in RPL than in any other type of PL. In these circumstances, identifying the genetic abnormality provides essential information on recurrence risk and helps prevent other kinds of potentially unnecessary evaluations and experimental treatments.

Conventional karyotyping has been widely applied from central laboratories to local hospitals, although two primary limitations have been noted. The first is that its requirement for cell culture, which may lead to prolonged turnaround time in case of culture failure. The second is the risk of sample cell contamination, resulting in approximately 29–58% of false-negative results [8,9].

Advanced and high-resolution methods, such as fluorescence in situ hybridization (FISH), array-based comparative genomic hybridization (aCGH), multiplex ligation-dependent probe amplification (MLPA), and next-generation sequencing (NGS), including whole-exome sequencing (WES), have overcome some of the disadvantages of conventional cytogenetic techniques and enabled the detection of aneuploidies, sub microscopic chromosomal imbalances, and mutations and polymorphisms.

This review aims to discuss the scientific advances in the genetic and/or genomic investigation of PL by paying attention to the specific type of abnormality.

Genetic Causes of PL

Aneuploidy

Chromosomal abnormalities are responsible for 70% of SPL and 30–50% of RPL cases [10]. Karyotyping the fetus is the first approach in such cases. These abnormalities are also common in loss with a combination of congenital malformations and can be found in 66% of anomalous embryos and 33% of anomalous fetuses [11]. Chromosomal changes were found in 90% of pre-embryonic loss, 50% of loss at 8–11 GW, 30% of loss at 16–19 GW, and only 6–12% of stillbirths [12,13]. The later the loss occurs, the lower the possibility that chromosomal change was involved.

Among all chromosomal disorders, aneuploidy is the most common cause for both sporadic and recurrent loss. Aneuploidy is defined as the state of having an abnormal number of chromosomes but not a whole multiple of the haploid number [14]. Epidemiological studies have shown that aneuploidy in the conceptus usually leads to SPL [15]. Interestingly, in women with RPL, if the prior loss is attributable to fetal aneuploidy, the subsequent loss is also likely to be aneuploid [16]. The most frequently found aneuploidies in the early loss were trisomies (72.4% in SPL and 52.5% in RPL), respectively trisomy 16 (10.6% of PL cases with trisomy), 8 (7.06% of PL cases with trisomy), and 20 (7.06% of PL cases with trisomy) [17]. Trisomies 21 (13.7% of cases with trisomy), 18 (8.9% of cases with trisomy), and 13 (7.0% of cases with trisomy) are mostly associated with late fetal loss [18,19]. Monosomy X was found to be involved in 10% of early fetal loss [20]. As aneuploidy can be identified by conventional karyotyping, some cases of the loss may be due to sub microscopic chromosomal changes, which require techniques with greater resolution [21].

Advanced maternal age is a major concern for oocyte aneuploidy. Recombination failure, cohesion deterioration, spindle assembly checkpoint dysregulation, abnormalities in post-translational modification of histones and tubulin, and mitochondrial dysfunction are the leading causes of oocyte aneuploidy associated with maternal aging [22]. Among both SPL and RPL cases, maternal age is associated with an increased risk of fetal aneuploidy [16]. Evidence suggests that women with RPL and advanced maternal age may benefit most from preimplantation aneuploidy screening [23].

Translocation

Chromosomal translocation is defined as the interchange of genetic materials between two non-homologous chromosomes [24]. Parental cytogenetic abnormalities are perhaps the most thoroughly investigated genetic causes of RPL. Microarray is believed to be reliable in detecting translocations. A study reported that microarray could yield results in 99.9% of fetal samples [25]. Microarray analysis of 3,975 tissue samples of the fetus from PL cases suggested that 1.3% (54 cases) harbor an unbalanced translocation in the fetus, with the potential of being inherited from a balanced carrier parent [26].

Approximately 2–5% of couples with RPL have been estimated to have a balanced reciprocal translocation [27]. The PL rate is higher in couples with the presence of a parental reciprocal chromosomal translocation than among couples with normal karyotypes [28].

Carriers of a balanced translocation may usually be phenotypically normal; their pregnancies are at greater risk of PL or may result in a live birth with multiple congenital malformations and/or intellectual disability secondary to a balanced chromosomal arrangement [29]. It is highly recommended to those couples in whom one partner is at risk of harboring a chromosomal translocation for proper genetic counselling with a translocation screening.

Copy number changes

High-resolution chromosomal microarray analysis (CMA) is a molecular technique that detects copy number variants (CNVs), sub microscopic gain or loss of DNA. Roughly 33% of RPLs were caused by CNVs, as reported by both Chinese and Estonian groups [30,31].

Applying CMA to analyze 5,507 cases of PL, it was found that the most prevalent pathogenic CNVs presented deletion at 22q11.21 or 1q36.33 [32]. The 22q11.2 deletion was screened in additional 22,451 conceptions of PL cases. Result gives an overall incidence of 1/1,497, suggesting this incidence was higher among PL cases than in the general population (1/4,000–1/6,000) [33]. An investigation with genome-wide single nucleotide polymorphism (SNP) high-resolution array discovered CNVs in 16.7% (10/60) of PL fetuses [34]. Using the same technique, scientists identified 396 CNVs in 101 euploid PL cases. Although they were all nonpahogenic, the size of the variant regions identified in PL ranged between 0.08 MB and 0.55 MB, and 93 genes were found in the region of CNV [35]. A large cohort of more than 7,000 samples of PL has been examed by aCGH, and the team discovered chromosomal aneuploidy in 53% of the loss; besides, sub microscopic abnormalities, including deletions, duplications, multiple regions of homozygosity, and variants of uncertain significance, were associated with another 5.08% of the PL. Most CNVs associated with PL have lengths ranging between 2 Mb and 400 kb [26]. The most common region of pathogenic CNVs was the highly imprinted region 11p15.5. This region is abundant with imprinted genes and has a vital role in the maternal-fetal exchange. Aberrant methylation or duplication of imprinted genes in this region could cause PL [34]. It is of particular interest that recurrent CNVs have been found to be associated with PL.

Mosaicism

Confined placental mosaicism (CPM) is defined as chromosomal differences between the fetus and placenta. It was first described by Warburton et al. in 1978 [36]. They reported that roughly 10% of trisomic conceptions contained a mosaic cell line. In later studies, this mosaicism was shown to be associated with an increased possibility of second- and third-trimester PL and intrauterine fetal growth retardation [37]. Over the entire gestational period, CPM can be found in over 2% of viable pregnancies [38]. Aneuploidy in a fetus may affect organ function, and placental aneuploidy also frequently leads to malfunction, resulting in growth retardation or even death from placental insufficiency. Early case-control studies have found CPM in more than 15% of growth-retarded fetuses [38].

Preimplantation genetic testing (PGT), which was originally described as aneuploidy screening to increase pregnancy rates, decrease loss rates, and establish transfer order, is increasingly utilized to supplement in vitro fertilization (IVF) [39]. NGS is a new and accurate tool in PGT for aneuploidy and is the only technique recommended by the Preimplantation Genetic Diagnosis International Society. A study compared various methods, including karyotyping, aCGH, and NGS, in an analysis of 38 fetal samples of PL. It was shown that aCGH could identify only 4 among 20 mosaic samples that were previously confirmed by NGS. This result indicated that NGS is a better technology in identifying mosaicism than aCGH [40]. It was also found that the rate of mosaicism was twice as high among embryos resulting in PL (12/38, 31.6%) than those resulting in live births (6/38, 15.8%) [40]. A rate of 55.6% PL in blastocysts was classified as mosaic versus 17.2% for euploid control samples [41]. PL rates were significantly lower after euploid embryo transfer than with mosaic (containing 20-80% abnormal cells) embryo transfer [42]. It appeared that the degree of mosaicism of trophectoderm from blastocysts is an ideal predictor of ongoing pregnancy and miscarriage [33]. Controversially, after evaluating the pregnancy outcomes of 143 mosaic and 1,045 euploid embryos, the research team found that the degree of mosaicism in trophectoderm could not predict pregnancy potential [43].

Mutations and single nucleic variations

Several inborn metabolic errors, hemoglobinopathies, and X-linked disorders are associated with PL. Severe untreated α-thalassemia may lead to universal fetal loss [44]. Most of the mutations or SNPs found in aborted fetuses were inherited from one or both side(s) of the parents. The first application of NGS in this field was published in 2013 by Shamseldin et al. [45]. This team examined a family with RPL due to non-immune hydrops fetalis by exome sequencing and identified a novel missense mutation in CHRNA1 responsible for this medical condition [45]. The same group collected samples of 24 consanguineous families with RPL due to lethal non-immune hydrops fetalis and then screened them with exome sequencing. Possible pathogenic homozygous mutations were identified in seven genes. Recurrent mutations in this cohort were detected in one of the genes (THSD1) with a role in angiogenesis and maintenance of vascular integrity [46]. In 2018, the team studied 44 more families with lethal pregnancy outcomes. Pathogenic variants were observed in 50% of these families, and variants of unknown significance (VUS) were found in 34% of these families. The mutations in all cases except for one were homozygous and predominantly missense. VUS included genes known to be responsible for postnatal disorders (phenotype expansion) and genes not previously associated with human disease [47].

WES was applied in studying 19 unrelated conceptuses from very early spontaneous abortion from non-consanguineous couples with no previous successful pregnancy [38]. Bioinformatics analysis was introduced to variants from a list of 286 selected candidate genes associated with early embryonic lethality. Thirty-six sequence variants from 32 genes were associated with the loss in 15 of 19 patients. Further studies by in silico bioinformatics showed that the LIM domain-binding protein 1 (c.662C>T; p.S221L) variant was a highly pathogenic variant [48]. Another WES study of a family with RPL identified a compound heterozygous of a deletion and a nonsense mutation in the gene KIF14 from both lost fetuses. Each was contributed from one side of the parents [49].

In 2015, a family with severe spontaneous PL has been carefully studied [40]. Both cytogenetic and molecular genetic approaches had been performed on the female proband and her mother, each of whom had had 18 times of loss. Triploids was found in six screened products of loss. Microsatellite analysis illustrated that triploids was a result of maternal meiosis II error. An autosomal dominant mutation affecting meiosis in the proband and her mother was proposed to contribute to recurrent triploidy. WES identified mutations in 47 genes shared between the mother and daughter. Eight genes, including PLCD4 and OSBPL5, coded for proteins implicated in oocyte maturation/activation and polar body extrusion. These mutations were candidate variants for recurrent triploidy in females in this family [50].

2017 a WES study was performed for 49 unrelated women with early RPL [51]. The study focused on 234 PL candidate genes preselected based on previous systematic literature reviews, endometrium expression, and murine models. The products of these genes are involved in many processes considered relevant for fetal maintenance and health. Twenty-seven mutations in the coding region of 22 genes were found in 20/49 female participants (41%) and were considered potentially causative for the phenotype based on rarity, type of mutation, and conservation. Seventeen mutations in 16 genes were bioinformatically evaluated to be pathogenic. The functions of these gene products were related to cell adhesion-trophoblast endometrium interaction, coagulation, extracellular

matrix remodeling, angiogenesis, cell proliferation, differentiation, migration, apoptosis, metabolism, and immunological function modulation. Functional and structural analyses were also done for two of the mutated genes, FGA and MMP10, and showed significant changes in protein stability secondary to FGA-p. Phe685Cys and MMP10-p.Asp199Asn mutations, which strongly suggest a deleterious effect leads to RPL. In a family with very early RPL, a compound heterozygous mutation was detected in two miscarriages by WES. The gene harboring the mutation, DYNC2H1, is known to cause lethal fetal akinesia. Mutations in DYNC2H1 are typically reported in later pregnancies, and the mutations identified in embryonic losses in this family expand the phenotype associated with this gene [51]. Similar investigations have been launched. Several mutations/variants have been identified in various genes, including AMN, STAT3, PROCR, VEGF, TP53, and NOS3, and have been associated with SPL [52-54]. These affected genes have been found to participate in a variety of biological processes, including ciliogenesis, intra-flagellar transport, RNA transport and processing, and the cholinergic signaling pathway.

Epigenetic studies

Epigenetic factors are important for maintaining correct gene expression to ensure cellular and tissue homeostasis, especially during various reproduction processes, including meiosis, embryo development, implantation, tissue remodeling, and pregnancy maintenance. Dysregulation in epigenetic mechanisms may lead to disturbances in the normal biological process and result in many diseases, such as uncertain RPL. Although not much information is available about the participation of epigenetic alterations in multiple PL, these changes have been implicated in reproductive complications [55]. A study based on Mexican couples with three or more losses identified heterochromatin polymorphism in 29.1% of the male member and 21.5% of the female member of the couples [56].

Among the different alterations, the unbalanced inactivation of maternal and paternal X chromosomes in women (also known as skewed X chromosome inactivation, SXCI) has been associated with RPL. Somatic cells from female mammals contain two X chromosomes, one of which is randomly inactivated during the embryonic period, resulting in one functional X chromosome in all cells throughout life [57]. In normal females, the inactivation occurs randomly so that each X chromosome (maternal or paternal) remains active in approximately 50% of somatic cells. There are widely accepted evidence about the association between SXCI and PL, but some studies fail to demonstrate such data. The results of one study that compared the X-chromosome inactivation pattern of 357 women who had had two or more spontaneous losses showed no association between SXCI and PL [58]. Another multicenter study analyzed 101 pairs of women with RPL and healthy controls and found that SXCI status does affect pregnancy outcome [59]. To minimize the bias in statistics and analysis, in 2015, a meta-study reviewed 12 previous investigations of 1,594 RPL patients and matched 1,924 healthy controls. Results showed that extreme SXCI (defined as SXCI occurring in 90–95% of the cells) was associated with RPL in women with three or more losses [60]. However, SXCI was significantly and consistently associated with RPL, found by Su et al. [61].

There has been an increased effort in research on RNAs in the last few years, such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs). These nucleic acids were shown to play an important role in gene expression regulation. In 2014, a Korean group identified that miRNAs rs3742330, rs10719, rs11077, and rs14035 might contribute to the development of RPL via regulating the expression of their host genes DICER, DROSHA, XPO5, and RAN [62]. As all of the product of the above genes have RNA binding activity, this might be the very first research showed one of the potential mechanisms of epigenetic regulation involved in the pathogenesis of RPL. More studies have shown the associations between miRNAs and related pregnancy adverse outcomes. Polymorphisms of rs12976445 and rs41275794 in pri-miR-125a alter mature miRNA expression and associate with RPL in the Chinese population [63]. Functional investigation illustrated that mutant pri-miR-125a can disturb the expression of miR-125a targetome and then enhance the invasive capacity of endometrial stromal cells (ESCs) and increase the sensitivity of ESCs to mifepristone [64]. Furthermore, the miRNA-regulated ubiquitin pathway was also found to participate in the pathogenesis of RPL by inhibiting trophoblast migration and invasion [65].

LncRNAs are other factors of interest in the epigenetic control of gene expression. Research on these RNAs also contributes to understanding the mechanisms involved in PL, especially the recurrent type. Evidence was found that lncRNA regulated infection and inflammation pathways associated with PL [66]. The analysis of chorionic villi by lncRNA array identified 1,449 lncRNAs differentially expressed between RPL and healthy controls [67]. Recently, altered expression of epigenetic regulators and imprinted genes in human placenta and fetal tissues have been discovered from second-trimester SPL [68].

Conclusion

PL is a complex disorder influenced by both genetic and environmental factors. Management of RPL depends on the identified cause. Conventional karyotyping is the gold standard for a major chromosomal rearrangement and detection of polyploid, whereas aCGH, WES can detect other genetic deficits, including minor chromosomal changes and mutations.

For couples with recurrent aneuploidy or unbalanced losses, the available options include IVF/PGT or expectant management. PGT-aneuploid with selection and transfer of a euploid embryo has

been shown to significantly decrease the risk of subsequent PL, but further investigation of this emerging strategy is necessary before daily adoption in the RPL population. Although WES is costly to be applied routinely in clinical diagnosis of RPL, the identification of genes associated with RPL will be favorable for patients after further treatment and the availability of PGT.

Great strides have been made to increase the resolution and throughput to discover the genetic causes of PL, and individual factors have limited the quality of these studies. The first limitation is sample size: the number of cases/families with PL investigated so far is still too small to allow the identification of mutations or affected genes. Much larger and more comparable (especially in clinical signs) cohort studies are required in all these areas to determine the weight of candidate genes and to separate these functional contributions to the condition. The lack of detailed phenotypes in early PL can only be addressed by transcervical embryoscopy or transvaginal ultrasound in intact recurrent PL. Some studies included information about cases such as scan abnormalities or post-mortem abnormalities of losses and hystero-embryoscopy as supplements to correlate with genetic results. These studies were difficult to compare, as the standards and definitions were unmatched between the cohorts studied and the methods of evaluation.

Future efforts should be towards increasing the sample sizes of patients affected by PL (and their families), preferentially couples with multiple losses, detailed medical descriptions of the phenotypes and pathology of the loss, and couples' obstetric history. It would also be encouraging to use the latest bioinformatic algorithms to interpret and analyze the massive amount of data gathered from NGS. Such efforts will improve our understanding of the causes of PL and facilitate the management of the condition, leading to successful pregnancy outcomes for families that have experienced PL.

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