Reproductive Health

Human Reproduction Archives

ISSN 2595-0711

SYSTEMATIC REVIEW

Copy number variations as a risk factor for couples with idiopathic recurrent miscarriage: A systematic review

Sheila Janaina Sestari[1*], Bruno Faulin Gamba[1], Francis Patrício França Ferreira[1], Roberta Machado de Oliveira Frota Curado[1], Eduardo Camelo de Castro[2], Nádia Aparecida Bérgamo[1], Lucilene Arilho Ribeiro-Bicudo[1]

[1]Universidade Federal de Goiás (UFG), Instituto de Ciências Biológicas, Departamento de Genética, Laboratório de Genética Molecular e Citogenética, Goiânia, Goiás, Brasil

[2]Hospital das Clínicas da Universidade Federal de Goiás, Departamento de Reprodução Humana, Goiânia, Goiás, Brasil

Abstract

Objective: It is well established that inherited chromosomal alterations such as copy number variations (CNVs) are associated to miscarriage. However, most studies focus on evaluating CNVs only in products of conception. The aim of this systematic review was to highlight the importance of investigating CNVs in couples with a history of recurrent miscarriage as well as their role in pregnancy, filling part of the gap between studies of recurrent miscarriage. **Methods**: A search in PubMed, Scientific Electronic Library Online (SCIELO), Latin American and Caribbean Literature in Health Sciences (LILACS), and Portal de CAPES/MEC databases for relevant published articles was conducted using the following controlled search terms: "copy number variation", "cnv", "miscarriage", "recurrent miscarriage", "spontaneous abortion", "loss pregnancy", "couple", "microarray analysis", "comparative genomic array", and "array CGH". The Boolean operators AND and OR were used. The search captured studies published up to October 2020. **Results**: A total of five studies were extracted for the present analysis. Sixteen CNVs involving the PDZD2, GOLPH3, TIMP2, CTNNA3, STS, EGFL6, STX6, CETN2, CTDSPL, GSTT1, HLA, MSR1, NIPA1, NIPA2, CYFIP1 and TUBGCP5 genes on ten different chromosomes were considered at potential risk for pregnancy maintenance. **Conclusion**: The findings of the present study affirm the importance of investigating the role of CNVs in couples with recurrent miscarriage and not just products of conception, in addition contributing to more accurate medical diagnoses.

Keywords: copy number variations, recurrent miscarriage, chromosomal rearrangement, abortions, pregnancy maintenance.

Introduction

Miscarriage is common during pregnancy, occurring in 15% of all clinically recognized pregnancies. This frequency is higher when associated with advanced maternal age[1]

There are two types of miscarriage: sporadic and recurrent. About 25% to 50% of couples experience one or more sporadic miscarriage.[1] By contrast, only about 3% of couples undergo recurrent miscarriage (RM).[2] Although RM is experienced by a smaller percentage of couples, its etiology is complex and the search for diagnoses and treatments has given rise to different findings for this group.[3,4] In addition, it has been established that the greater the number of abortions, the greater the probability of the next occurrence.[5,6]

RM is thus defined as the condition in which losses are consecutive and occur until the 22nd week of gestation. However, to date, no consensus exists regarding the number of abortions that constitutes RM.[7]

According to the guidelines for the investigation and treatment of couples with RM, the Royal College of Obstetricians and Gynecologists define the condition as three or more consecutive spontaneous abortions, whereas the European Society for Human Reproduction and Embryology uses the definition of loss of two or more pregnancies. The American Society for Reproductive Medicine uses the definition of two or more consecutive spontaneous abortions, although it

Conflicts of interests: The authors have no conflicts of interest to declare.
Submitted: November 23, 2020
Accepted: July 15, 2022
Study carried out at Laboratory of Molecular Genetics and Cytogenetics, Institute of Biological Sciences, Federal University of Goias (UFG), Goiânia, GO, Brazil.

recommends that in epidemiological studies, only couples with three or more spontaneous abortions be included and emphasizes that biochemical losses should not be included in the group of RM.[8-10] The etiology of RM includes a spectrum of factors, such as uterine malformations, endocrinological and immunological causes, as well as thrombophilic, genetic, and lifestyle factors like alcohol and tobacco consumption. It is worth highlighting that despite extensive evaluations, ~50% of RM have no identifiable factors and are classified as idiopathic RM.[11]

Genetic factors contribute to a significant fraction of pregnancy losses, particularly chromosomal alterations such as deletions and duplications.[12,13] These alterations are commonly noted through conventional karyotyping, and their identification is limited to changes larger than 5 Mb. With the advancement of DNA technologies, such as array-comparative genomic hybridization (CGH), submicroscopic deletions and duplications, which are also known as copy number variations (CNVs), could be identified and associated with several disorders, including RM.

CNVs are deletions or duplications of >50 bp DNA sequences that disrupt about 13% of RefSeq genes.[14-17] They are mainly formed by errors in DNA replication and spontaneous and/or induced mutations and are responsible for about 17.7% of the variabilities in gene expression.[14,17-22]

Currently, there are several studies indicating the association of CNVs with RM, but the vast majority only evaluated CNVs in products of conception (POCs) and not in parents.[23-28] However, it is known that inherited CNVs can lead to RM if the CNV contains printed genes (expressed from only one parent in the tissues of pregnancy), if the CNV has one or more genes that are relevant to embryonic/placental growth and has a mutation in the other allele, or if one or more CNV genes are expressed in a variable way.[29]

Therefore, the purpose of this paper is to highlight the importance of investigating CNVs in couples with RM history as well as their role in pregnancy, since CNVs may carry genes that impact embryogenesis or maintenance of pregnancy. Thus, we seek to contribute with new information on the genetic factors that corroborate RM.

Methods

This systematic review has been registered with PROSPERO (CRD42020201855). This research was developed through a systematic literature review to assess the studies investigating the contribution of CNVs to RM in couples with a history of at least two gestational losses during the first trimester of pregnancy and conducted according to the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

Search Strategy

Publications in the PubMed, Scientific Electronic Library Online (SCIELO), Latin American and Caribbean Literature in Health Sciences (LILACS), and Portal de CAPES/MEC databases were identified using the following controlled search descriptors: “copy number variation”, “cnv”, “miscarriage”, “recurrent miscarriage”, “spontaneous abortion,” “loss pregnancy”, “couple”, “microarray analysis”, “comparative genomic array”, and “array CGH”. The Boolean operators AND and OR were used, taking into account the particularities, characteristics, and filters of each of the selected databases. The search captured studies published up to October 2020. Appendix A (Table A1) contains the corresponding search terms and MeSH terms.

Eligibility Criteria

No language or date restrictions were applied, and the search was conducted until October 2020. Studies assessing the analysis of CNVs in couples and women with a history of 2 or more consecutive idiopathic miscarriages up to the 22nd week of pregnancy. The study designs considered were analytical observational: cohort, cross-sectional, and case and control studies in which CNV analyses were performed in those individuals.

Study Selection and Data Extraction Process

The selection of articles used in this study was carried out by two independent reviewers. From the initial search, the procedure included the reading of the titles/abstracts and the exclusion of duplicate articles. The summaries were read, followed by the full texts, taking into account the established eligibility criteria. Depending on each study design, a checklist was formulated to assess the methodological quality of each of the selected articles. Studies that identified CNVs only in POCs were excluded. The details of the criteria used for inclusion and exclusion of studies are presented in Appendix A (Table A2).

The following data were extracted for each study included in this systematic review: authors, date of publication, country, CNV, type of CNV, cytogenetic location, main genes with potential risk of RM, and gene function (shown in Table 1).

Table 1. Summary of studies that analyzed CNVs in couples with RM.

Author, Date and Country	Gene	Locus	CNV/ Type	Function Gene
Nagirnaja et al., 2014 *Tartu, Estonia*	*PDZD2, GOLPH3*	5p13.3	Gain/U	PDZD2: function poorly defined and hasmainly addressed as a tumor supressor; GOLPH3: essential for Golgi trafficking and maintenance of its structure. Its genomic amplification is linked to oncogenic features and activation of the signaling pathway of mechanistic target of rapamycin (mTOR).
Rajcan-Separovic et al., 2010 *Vancouver, Canada*	*TIMP2*	17q25.3	Gain/U	Inhibitor of trophoblast invasion. *Complexes with metalloproteinases (such as collagenases) and irreversibly inactivates them by binding to their catalytic zinc cofactor.
	CTNNA3	10q21.3	Loss/C	Inhibitor of trophoblast invasion. *May be involved in formation of stretch-resistant cell-cell adhesion complexes.
	STS	Xp22.31	Gain/C	Strong expression in the placenta in normal pregnancies. Uterine receptivity.
	EGFL6	Xp22.2	Gain/U	Strong expression in the placenta in normal pregnancies. Estrogen production. *May bind integrin alpha-8/beta-1 and play a role in hair follicle morphogenesis. Promotes matrix assembly.
Rajcan-Separovic et al., 2009 *Vancouver, Canada*	*STX6*	1q25.3	Loss/U	Endosome organization and biogenesis, cell adhesion, intracellular transport
	CETN2	Xq28	Gain/U	Centrosome component, sub-unit of nuclear pore, mRNA and protein transport
Karim et al., 2017 *Jeddah, Saudi Arabia*	*CTDSPL*	3p22.2	Gain/U	Phosphatase activity to dephosphorylate the C-terminal domain of RNA polymerase II and regulates cell growth and differentiation; regulates chromosomal recombination and segregation, and chromosomal synapsis by encoding components of the synapto-nemal complex.
	GSTT1	22q11.23	Gain/U	Crucial for endometrial differentiation and embryonic growth.
	HLA	6p21.33	Loss/U	Role in allograft rejection, additional studies suggested them as risk alleles for unexplained recurrent abortion.
	MSR1	8p22	Gain/U	Regulate maternal immune tolerance by activating T cell response through uterine dendritic cells
Kasak et al., 2017 *Tartu, Estonia*	*CTNNA3*	10q21.3	Loss/C	Inhibitor of trophoblast invasion. *May be involved in formation of stretch-resistant cell-cell adhesion complexes.
	NIPA1, NIPA2, CYFIP1, TUBGCP5	15q11.2	Gain/C	**NIPA1*: acts as a Mg2+ transporter. Can also transport other divalent cations such as Fe2+, Sr2+, Ba2+, Mn2+ and Co2+ but to a much less extent than Mg2+; **NIPA2:* acts as a selective Mg^{2+} transporter; **CYFIP1:* Component of the CYFIP1-EIF4E-FMR1 complex which binds to the mRNA cap and mediates translational repression. Regulates formation of membrane ruffles and lamellipodia. Plays a role in axon outgrowth. May act as an invasion suppressor in cancers; **TUBGCP5:* gamma-tubulin complex is necessary for microtubule nucleation at the centrosome.

CNV, copy number variation; U, unique; C, common; p, short arm; q, long arm. * UniProt data access: uniprot.org

Quality Assessment

The quality of each study was assessed using a modified Newcastle-Ottawa scale (NOS) evaluation method (shown in Appendix A (Table A3)). Each study was evaluated by sample size, inclusion/exclusion criteria, methodology used, statistical analysis, case definition, controls, and comparability, with a maximum score of 10 points (shown in Appendix A (Table A4)). High-quality articles with an NOS score of ≥ 7 were included in this study.

Results

Summary of Study Characteristics

A total of five studies that assessed the association of CNVs in couples with RM history were included in this systematic review. The PubMed, SCIELO, LILACS, and Portal de CAPES/MEC databases identified 2029 articles associated with the topic, of which 230 were duplicated and then removed. After screening the titles and abstracts, 27 articles were pooled for detailed analysis, but 22 of them were excluded because they did not meet the inclusion criteria. The process applied in the study selection is shown in Figure 1.

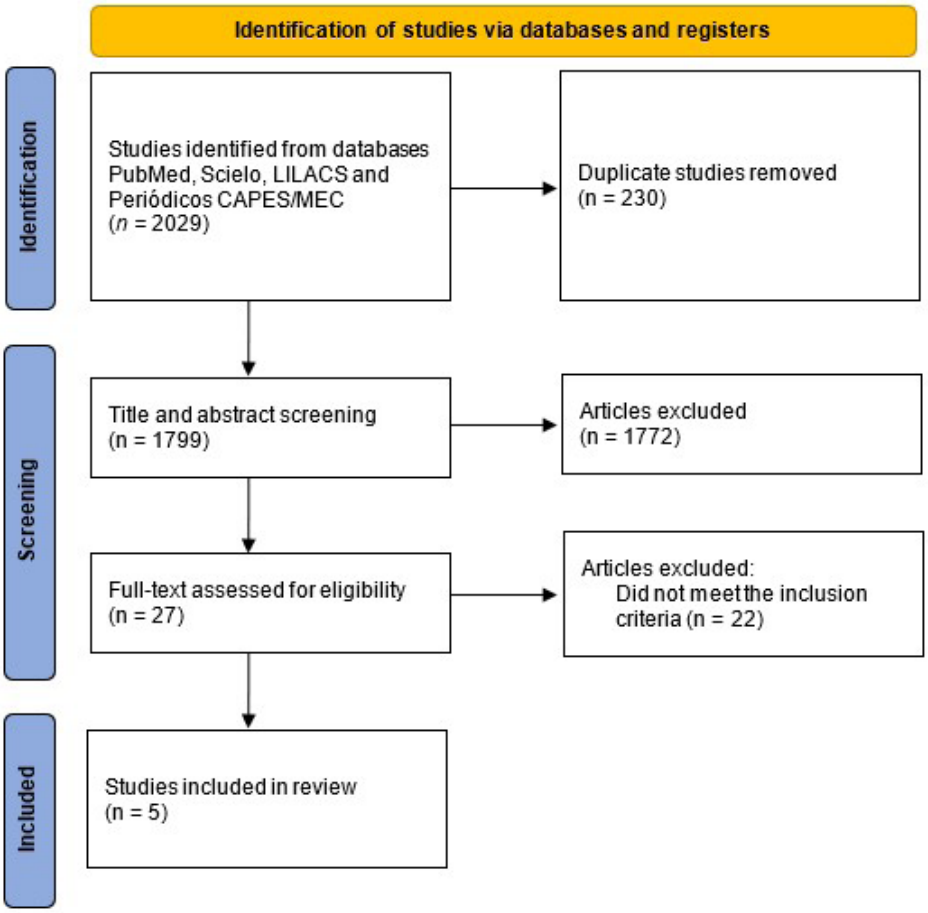

Fig. 1. PRISMA flow diagram of information through the different phases of the systematic review

The analyzed studies were all published in English between 2010 and 2017 and identified a total of 1409 CNVs in couples and women with idiopathic RM. Sixteen out of the 1409 CNVs were considered to have a potential risk for RM due to the presence of rearranged genes that are important for the maintenance of pregnancy (shown in Fig. 2). Meanwhile, the rest of the CNVs were classified as variants of common or unknown significance.

CNVs Harmful to Pregnancy

In this systematic review, based on the literature, we identified a total of 1409 CNVs in couples and women with idiopathic RM. Sixteen out of the 1409 CNVs were identified on 10 different chromosomes and considered to have a potential risk for RM due to the presence of rearranged genes that are important for the maintenance of pregnancy (shown in Fig. 2). Meanwhile, the rest of the CNVs were classified as variants of common or unknown significance. The main characteristics of the five studies included in this systematic review are outlined in Table 1 (author, date, country, gene, *locus*, CNV/type, and function gene).

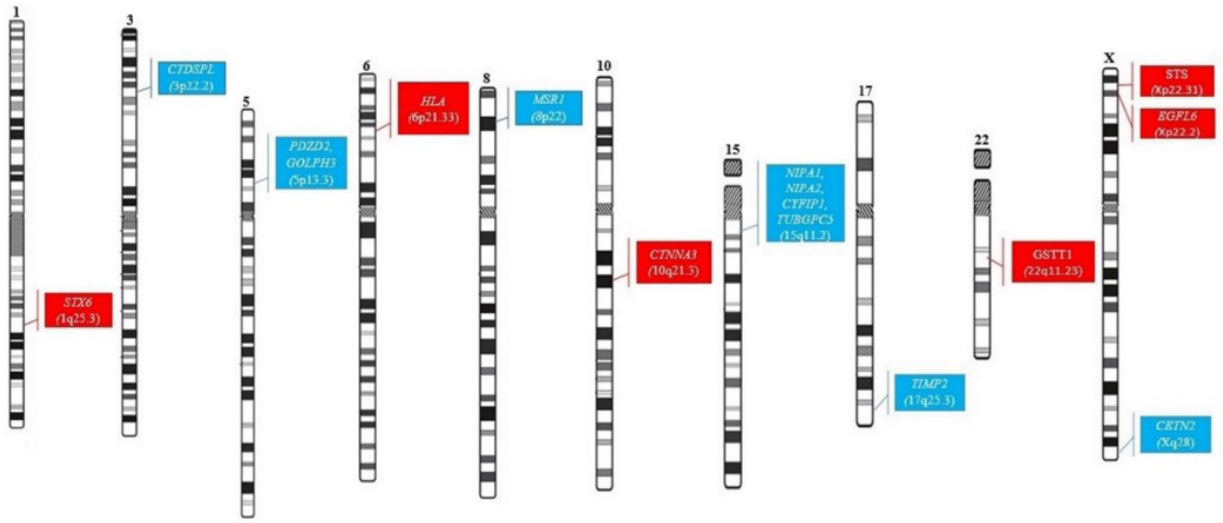

Fig. 2. Chromosomal distribution of CNVs in couples with idiopathic RM. Boxes indicate locations of duplication (blue) and deletion (red) CNVs and genes covered. p, short arm; q, long arm

Discussion

CNVs can affect the dosage of genes that are critical for early pregnancy or disrupt normal chromosomal segregation, possibly leading to aneuploidy[30]. When inherited from unaffected parents, CNVs are generally considered to be benign. However, they can be harmful to pregnancy due to epigenetic factors, variable expression, or unmasking of recessive alleles, even if they do not alter the parents' phenotype.[29]

CNVs and the Genes Covered

A deletion in the 10q21.3 region including the *CTNNA3* gene was identified in two independent studies involving a mother with a history of RM and her POC, as well as the placental genome of an RM case.[30,31] *CTNNA3*, normally expressed only from the maternal allele in the placenta, encodes the αT-catenin protein, a cell adhesion molecule that regulates the balance between the proliferation and invasion of trophoblasts.[32] Notably, it has been established that the invasion of trophoblasts in the endometrial stroma and internal third of the myometrium during gestational development is essential for the definitive maternal-fetal circulation and for the success of the pregnancy.[33]

Like *CTNNA3, TIMP2* acts as a trophoblast invasion inhibitor[34,35] that has a functional role in early pregnancy homeostasis.[36] A 17q25.3 duplication involving the *TIMP2* gene was identified in a woman with a history of five RM and inherited at 4/5 RM. Rearrangement was associated with two hypotheses, namely, impaired development of the placenta or impaired remodeling of the endometrium at the beginning of pregnancy, both of which have the potential to lead to RM.[31] In addition, it has been observed that women with RM have a high serum level of *TIMP2*, thus increasing the risk of miscarriages.[36]

Rajcan-Separovic et al.[31] identified two CNVs involving the X chromosome in women with RM and their POCs. In the Xp22.2 region, a duplication involving the *EGFL6* gene was observed, while in Xp22.31, a deletion was identified covering the STS gene. Both genes are strongly expressed in the placenta in healthy pregnancies and play important roles in maternal reproductive tissues during pregnancy, such as uterine receptivity and estrogen production; therefore, they can be considered candidate genes for RM when altered.[37-39]

Chromosome 5 (5p13.3), a duplication *PDZD2:GOLPH3*, was identified in two sets of independent samples from northern European populations with a frequency that is five times higher in RM women compared with the fertile control group or the world population.[16] This CNV does not directly alter the number of copies of the entire coding regions of the genes, but it can interfere with transcription and deregulate the involved or neighboring genes.[40,41] The *PDZD2:GOLPH3* duplication has also been identified as a risk factor for preeclampsia.[42]

The expression profile of *PDZD2* and *GOLPH3* in human tissue panels reveals mutually elevated levels of transcription in the placenta and ovary, thus supporting the functional relevance of rearrangement during pregnancy. In addition, *GOLPH3* is responsible for activating the mechanistic targeting pathway of rapamycin (mTOR).[43] Notably, changes in mTOR signaling are associated with several reproductive disorders, including RM.[44-48]

Karim et al.[49] identified CNVs by disrupting the *GSTT1, MSR1, CTDSPL*, and *HLA* genes in RM couples. The *GSTT1* gene located in the 22q11.23 region showed a significant duplication. It is known that this gene is essential for endometrial differentiation and embryonic growth and that its overexpression is associated with RM.[50] Changes in the *MSR1* gene are also associated with RM due to its role in regulating maternal immune tolerance.[51] In turn, duplications involving the *CTDSPL* gene were identified in 39% of the RM couples analyzed. This gene is responsible for regulating recombination and segregation and chromosomal synapse. Changes in this gene are also associated with RM susceptibility independently[49] or by interaction with the *SYCP3, RB, SMAD*, and *TNF-beta* genes.[33,52,53]

In addition to the identification of CNVs, Karim et al.[49] analyzed the enrichment of the signaling pathways of genes affected by them and found that the locus of the *HLA* gene present in the 6p21.32 region was doubled in half of the analyzed cases. Changes in this gene can cause an immune response in the mother's body, allowing white blood cells to attack new embryo cells and lead to RM.[54-56]

According to the literature, large CNVs increase the probability of alterations in the key candidate genes or pathways involved in maintaining early pregnancy, thereby resulting in RM.[57,58] Kasak et al.[30] identified a 500-kb pericentromeric duplication in the 15q11.2 region in a male partner involving the *NIPA1, NIPA2, CYFIP1*, and *TUBGCP5* genes. CNVs in 15q11 have been reported as a common finding in cases of euploid RM.[25] Substantial rearrangements in the pericentromeric and subtelomeric regions can interfere with correct chromosomal pairing and segregation during meiosis and mitosis, leading to unviable offspring.[30]

Rajcan-Separovic et al.[59] identified two CNVs in a couple with a history of three RMs. An analysis of one of the couple's embryos showed that both were inherited. The woman had a 1q25.3 deletion interrupting the *STX6* gene, while her spouse had an Xq28 duplication involving the *CETN2* gene. It has been established that dysfunction in the *STX6* gene can lead to cell death[60] and that its knockdown can inhibit cell adhesion, a crucial process for embryonic development.[61] The *CETN2* gene plays an important role during the duplication of centrioles in the S phase and their separation in mitosis.[62] The transmission of paternal centrosome and CNV involving *CETN2* possibly leads to RM since the absence or even the alteration of centrosomes may result in embryonic abortion or infertility.[63,64]

CNV identification is dependent on the use of cytomolecular methods, such as medium to high density array CGH and array SNP. Analyzes based on microdeletions/microduplications microarrays, in addition to conventional cytogenetics, allow the identification of an "unfavorable" genome in one or both partners, allowing genetic counseling and appropriate clinical management.[30]

Our findings showed that the 16 CNVs cited above act as probable risk factors for RM. This evidence constitutes an alert in clinical practice since the routine examination for the detection of chromosomal abnormalities in couples and/or women with a history of RM is the karyotype. It is worth mentioning herein that although the karyotype is fundamental in detecting balanced rearrangements, it is inefficient for detecting small CNVs, such as those pointed out in this study.

This study has some limitations, including the limited data from analyzes of CNVs in couples with RM. This could be partially related to investigation of CNVs, mostly in POCs, as well as the lack of consensus regarding the number of miscarriages to define the RM.

In conclusion, RM is a complex condition that requires etiological consideration for the performance of appropriate management. Therefore, more studies identifying CNVs in couples and not just in the POCs are necessary because although CNV may not be expressed in parents, it can be expressed in pregnancy.

Acknowledgements

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG) by grant awarded.

References

1. Rai R, Regan L. Recurrent miscarriage. Lancet. 2006;368(9535):601-11. **http://dx.doi.org/10.1016/S0140-6736(06)69204-0**. PMid:16905025.
2. Christiansen OB, Steffensen R, Nielsen HS, Varming K. Multifactorial etiology of recurrent miscarriage and its scientific and clinical implications. Gynecol Obstet Invest. 2008;66(4):257-67. **http://dx.doi.org/10.1159/000149575**. PMid:18679035.
3. Alijotas-Reig J, Garrido-Gimenez C. Current concepts and new trends in the diagnosis and management of recurrent miscarriage. Obstet Gynecol Surv. 2013;68(6):445-66. **http://dx.doi.org/10.1097/OGX.0b013e31828aca19**. PMid:23942472.
4. van den Berg MMJ, van Maarle MC, van Wely M, Goddijn M. Genetics of early miscarriage. Biochim Biophys Acta. 2012;1822(12):1951-9. **http://dx.doi.org/10.1016/j.bbadis.2012.07.001**. PMid:22796359.
5. Barini R, Couto E, Mota MM, Santos CTM, Leiber SR, Batista SC. Fatores associados ao aborto espontâneo recorrente. Rev Bras Ginecol Obstet. 2000;22(4):217-23. **http://dx.doi.org/10.1590/S0100-72032000000400005**.

6. Ogasawara M, Aoki K, Okada S, Suzumori K. Embryonic karyotype of abortuses in relation to the number of previous miscarriages. Fertil Steril. 2000;73(2):300-4. **http://dx.doi.org/10.1016/S0015-0282(99)00495-1**. PMid:10685533.
7. Sugiura-Ogasawara M, Ozaki Y, Suzumori N. Management of recurrent miscarriage. J Obstet Gynaecol Res. 2014;40(5):1174-9. **http://dx.doi.org/10.1111/jog.12388**. PMid:24754846.
8. Royal College of Obstetricians & Gynaecologists. The investigation and treatment of couples with recurrent first-trimester and second-trimester miscarriage [Internet]. RCOG: London; 2011 [cited 2020 Nov 23]. (Green-Top Guideline; No. 17). Available from: https://www.rcog.org.uk/media/3cbgonl0/gtg_17.pdf
9. Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, Middeldorp S, et al. ESHRE guideline: recurrent pregnancy loss. Hum Reprod Open. 2018;2018(2):hoy004. PMid:31486805.
10. Practice Committee of the American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril. 2012;98(5):1103-11. **http://dx.doi.org/10.1016/j.fertnstert.2012.06.048**. PMid:22835448.
11. Christiansen OB, editor. Recurrent pregnancy loss. Oxford: John Wiley & Sons; 2013. **http://dx.doi.org/10.1002/9781118749012**.
12. Hassold TJ, Jacobs PA. Trisomy in man. Annu Rev Genet. 1984;18(1):69-97. **http://dx.doi.org/10.1146/annurev.ge.18.120184.000441**. PMid:6241455.
13. Kalousek DK, Langlois S, Barrett I, Yam I, Wilson DR, Howard-Peebles PN, et al. Uniparental disomy for chromosome 16 in humans. Am J Hum Genet. 1993;52(1):8-16. PMid:8434609.
14. Conrad DF, Pinto D, Redon R, Feuk L, Gokcumen O, Zhang Y, et al. Origins and functional impact of copy number variation in the human genome. Nature. 2010;464(7289):704-12. **http://dx.doi.org/10.1038/nature08516**. PMid:19812545.
15. McCarroll SA, Kuruvilla FG, Korn JM, Cawley S, Nemesh J, Wysoker A, et al. Integrated detection and population-genetic analysis of SNPs and copy number variation. Nat Genet. 2008;40(10):1166-74. **http://dx.doi.org/10.1038/ng.238**. PMid:18776908.
16. Nagirnaja L, Palta P, Kasak L, Rull K, Christiansen OB, Nielsen HS, et al. Structural genomic variation as risk factor for idiopathic recurrent miscarriage. Hum Mutat. 2014;35(8):972-82. **http://dx.doi.org/10.1002/humu.22589**. PMid:24827138.
17. Zhang Y-X, Zhang Y-P, Gu Y, Guan F-J, Li S-L, Xie J-S, et al. Genetic analysis of first-trimester miscarriages with a combination of cytogenetic karyotyping, microsatellite genotyping and arrayCGH. Clin Genet. 2009;75(2):133-40. **http://dx.doi.org/10.1111/j.1399-0004.2008.01131.x**. PMid:19215247.
18. Feuk L, Carson AR, Scherer SW. Structural variation in the human genome. Nat Rev Genet. 2006;7(2):85-97. **http://dx.doi.org/10.1038/nrg1767**. PMid:16418744.
19. Freeman JL, Perry GH, Feuk L, Redon R, McCarroll SA, Altshuler DM, et al. Copy number variation: new insights in genome diversity. Genome Res. 2006;16(8):949-61. **http://dx.doi.org/10.1101/gr.3677206**. PMid:16809666.
20. Girirajan S, Brkanac Z, Coe BP, Baker C, Vives L, Vu TH, et al. Relative burden of large CNVs on a range of neurodevelopmental phenotypes. PLoS Genet. 2011;7(11):e1002334. **http://dx.doi.org/10.1371/journal.pgen.1002334**. PMid:22102821.
21. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, et al. Global variation in copy number in the human genome. Nature. 2006;444(7118):444-54. **http://dx.doi.org/10.1038/nature05329**. PMid:17122850.
22. Stranger BE, Forrest MS, Dunning M, Ingle CE, Beazley C, Thorne N, et al. Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science. 2007;315(5813):848-53. **http://dx.doi.org/10.1126/science.1136678**. PMid:17289997.
23. Sato T, Migita O, Hata H, Okamoto A, Hata K. Analysis of chromosome microstructures in products of conception associated with recurrent miscarriage. Reprod Biomed Online. 2019;38(5):787-95. **http://dx.doi.org/10.1016/j.rbmo.2018.12.010**. PMid:30926177.
24. Tekcan A, Elbistan M, Tural S, Çetinkaya MB. Effects of subtelomeric copy number variations in miscarriages. Gynecol Endocrinol. 2015;31(9):708-14. **http://dx.doi.org/10.3109/09513590.2015.1032929**. PMid:26291815.
25. Viaggi CD, Cavani S, Malacarne M, Floriddia F, Zerega G, Baldo C, et al. First-trimester euploid miscarriages analysed by array-CGH. J Appl Genet. 2013;54(3):353-9. **http://dx.doi.org/10.1007/s13353-013-0157-x**. PMid:23780398.
26. Wang H, Yuan D, Wang S, Luo L, Zhang Y, Ye J, et al. Cytogenetic and genetic investigation of miscarriage cases in Eastern China. J Matern Neonatal Med. 2020;33(20):3385-90. **http://dx.doi.org/10.1080/14767058.2019.1572738**. PMid:30741046.
27. Wang Y, Li Y, Chen Y, Zhou R, Sang Z, Meng L, et al. Systematic analysis of copy-number variations associated with early pregnancy loss. Ultrasound Obstet Gynecol. 2020;55(1):96-104. **http://dx.doi.org/10.1002/uog.20412**. PMid:31364215.
28. Wen J, Hanna CW, Martell S, Leung P, Lewis S, Robinson WP, et al. Functional consequences of copy number variants in miscarriage. Mol Cytogenet. 2015;8(1):6. **http://dx.doi.org/10.1186/s13039-015-0109-8**. PMid:25674159.
29. Lee C, Iafrate AJ, Brothman AR. Copy number variations and clinical cytogenetic diagnosis of constitutional disorders. Nat Genet. 2007;39(7, Suppl):S48-54. **http://dx.doi.org/10.1038/ng2092**. PMid:17597782.
30. 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. 2016;2017(7):1-12. PMid:28345611.
31. Rajcan-Separovic E, Diego-Alvarez D, Robinson WP, Tyson C, Qiao Y, Harvard C, et al. Identification of copy number variants in miscarriages from couples with idiopathic recurrent pregnancy loss. Hum Reprod. 2010;25(11):2913-22. **http://dx.doi.org/10.1093/humrep/deq202**. PMid:20847186.
32. van Dijk M, van Bezu J, van Abel D, Dunk C, Blankenstein MA, Oudejans CBM, et al. The STOX1 genotype associated with pre-eclampsia leads to a reduction of trophoblast invasion by α-T-catenin upregulation. Hum Mol Genet. 2010;19(13):2658-67. **http://dx.doi.org/10.1093/hmg/ddq152**. PMid:20400461.

33. Zhu Y, Lu Y, Zhang Q, Liu J-J, Li T-J, Yang J-R, et al. MicroRNA-26a/b and their host genes cooperate to inhibit the G1/S transition by activating the pRb protein. Nucleic Acids Res. 2012;40(10):4615-25. **http://dx.doi.org/10.1093/nar/gkr1278**. PMid:22210897.
34. Seval Y, Akkoyunlu G, Demir R, Asar M. Distribution patterns of matrix metalloproteinase (MMP)-2 and -9 and their inhibitors (TIMP-1 and TIMP-2) in the human decidua during early pregnancy. Acta Histochem. 2004;106(5):353-62. **http://dx.doi.org/10.1016/j.acthis.2004.07.005**. PMid:15530550.
35. Okamoto T, Niu R, Yamada S, Osawa M. Reduced expression of tissue inhibitor of metalloproteinase (TIMP)-2 in gestational trophoblastic diseases. Mol Hum Reprod. 2002;8(4):392-8. **http://dx.doi.org/10.1093/molehr/8.4.392**. PMid:11912288.
36. Anumba DO, El Gelany S, Elliott SL, Li TC. Circulating levels of matrix proteases and their inhibitors in pregnant women with and without a history of recurrent pregnancy loss. Reprod Biol Endocrinol. 2010;8(1):62. **http://dx.doi.org/10.1186/1477-7827-8-62**. PMid:20565712.
37. Bowen JA, Hunt JS. The role of integrins in reproduction. Proc Soc Exp Biol Med. 2000;223(4):331-43. **http://dx.doi.org/10.1046/j.1525-1373.2000.22348.x**. PMid:10721002.
38. Leon L, Bacallao K, Gabler F, Romero C, Valladares L, Vega M. Activities of steroid metabolic enzymes in secretory endometria from untreated women with Polycystic Ovary Syndrome. Steroids. 2008;73(1):88-95. **http://dx.doi.org/10.1016/j.steroids.2007.09.003**. PMid:17953976.
39. Yeung G, Mulero JJ, Berntsen RP, Loeb DB, Drmanac R, Ford JE. Cloning of a novel epidermal growth factor repeat containing gene egfl6: expressed in tumor and fetal tissues. Genomics. 1999;62(2):304-7. **http://dx.doi.org/10.1006/geno.1999.6011**. PMid:10610727.
40. Henrichsen CN, Chaignat E, Reymond A. Copy number variants, diseases and gene expression. Hum Mol Genet. 2009;18(R1):R1-8. **http://dx.doi.org/10.1093/hmg/ddp011**. PMid:19297395.
41. Henrichsen CN, Vinckenbosch N, Zöllner S, Chaignat E, Pradervand S, Schütz F, et al. Segmental copy number variation shapes tissue transcriptomes. Nat Genet. 2009;41(4):424-9. **http://dx.doi.org/10.1038/ng.345**. PMid:19270705.
42. Zhao L, Bracken MB, DeWan AT. Genome-wide association study of pre-eclampsia detects novel maternal single nucleotide polymorphisms and copy-number variants in subsets of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study cohort. Ann Hum Genet. 2013;77(4):277-87. **http://dx.doi.org/10.1111/ahg.12021**. PMid:23551011.
43. Tanwar PS, Kaneko-Tarui T, Zhang L, Teixeira JM. Altered LKB1/AMPK/TSC1/TSC2/mTOR signaling causes disruption of Sertoli cell polarity and spermatogenesis. Hum Mol Genet. 2012;21(20):4394-405. **http://dx.doi.org/10.1093/hmg/dds272**. PMid:22791749.
44. Vatin M, Burgio G, Renault G, Laissue P, Firlej V, Mondon F, et al. Refined mapping of a quantitative trait locus on chromosome 1 responsible for mouse embryonic death. PLoS One. 2012;7(8):e43356. **http://dx.doi.org/10.1371/journal.pone.0043356**. PMid:22916247.
45. Roos S, Jansson N, Palmberg I, Säljö K, Powell TL, Jansson T. Mammalian target of rapamycin in the human placenta regulates leucine transport and is down-regulated in restricted fetal growth. J Physiol. 2007;582(1):449-59. **http://dx.doi.org/10.1113/jphysiol.2007.129676**. PMid:17463046.
46. Leconte M, Nicco C, Ngô C, Chéreau C, Chouzenoux S, Marut W, et al. The mTOR/AKT inhibitor temsirolimus prevents deep infiltrating endometriosis in mice. Am J Pathol. 2011;179(2):880-9. **http://dx.doi.org/10.1016/j.ajpath.2011.04.020**. PMid:21718677.
47. Hirota Y, Cha J, Yoshie M, Daikoku T, Dey SK. Heightened uterine mammalian target of rapamycin complex 1 (mTORC1) signaling provokes preterm birth in mice. Proc Natl Acad Sci USA. 2011;108(44):18073-8. **http://dx.doi.org/10.1073/pnas.1108180108**. PMid:22025690.
48. Adhikari D, Zheng W, Shen Y, Gorre N, Hämäläinen T, Cooney AJ, et al. Tsc/mTORC1 signaling in oocytes governs the quiescence and activation of primordial follicles. Hum Mol Genet. 2010;19(3):397-410. **http://dx.doi.org/10.1093/hmg/ddp483**. PMid:19843540.
49. Karim S, Jamal HS, Rouzi A, Ardawi MSM, Schulten HJ, Mirza Z, et al. Genomic answers for recurrent spontaneous abortion in Saudi Arabia: an array comparative genomic hybridization approach. Reprod Biol. 2017;17(2):133-43. **http://dx.doi.org/10.1016/j.repbio.2017.03.003**. PMid:28431992.
50. Nair RR, Khanna A, Singh K. Association of GSTT1 and GSTM1 polymorphisms with early pregnancy loss in an Indian population and a meta-analysis. Reprod Biomed Online. 2013;26(4):313-22. **http://dx.doi.org/10.1016/j.rbmo.2012.12.004**. PMid:23433732.
51. Moldenhauer LM, Keenihan SN, Hayball JD, Robertson SA. GM-CSF is an essential regulator of t cell activation competence in uterine dendritic cells during early pregnancy in mice. J Immunol. 2010;185(11):7085-96. **http://dx.doi.org/10.4049/jimmunol.1001374**. PMid:20974989.
52. Wu Y, Evers BM, Zhou BP. Small C-terminal domain phosphatase enhances snail activity through dephosphorylation. J Biol Chem. 2009;284(1):640-8. **http://dx.doi.org/10.1074/jbc.M806916200**. PMid:19004823.
53. Sapkota G, Knockaert M, Alarcón C, Montalvo E, Brivanlou AH, Massagué J. Dephosphorylation of the linker regions of Smad1 and Smad2/3 by small C-terminal domain phosphatases has distinct outcomes for bone morphogenetic protein and transforming growth factor-β pathways. J Biol Chem. 2006;281(52):40412-9. **http://dx.doi.org/10.1074/jbc.M610172200**. PMid:17085434.
54. Krieg SA, Fan X, Hong Y, Sang QX, Giaccia A, Westphal LM, et al. Global alteration in gene expression profiles of deciduas from women with idiopathic recurrent pregnancy loss. Mol Hum Reprod. 2012;18(9):442-50. **http://dx.doi.org/10.1093/molehr/gas017**. PMid:22505054.
55. Mallia JV, Das DK, Maitra A. Role of HLA in human pregnancy. Int J Hum Genet. 2012;12(1):33-6. **http://dx.doi.org/10.1080/09723757.2012.11886159**.

56. Faridi RM, Agrawal S. Killer immunoglobulin-like receptors (KIRs) and HLA-C allorecognition patterns implicative of dominant activation of natural killer cells contribute to recurrent miscarriages. Hum Reprod. 2011;26(2):491-7. v**http://dx.doi.org/10.1093/humrep/deq341**. PMid:21159685.
57. Pinkel D, Albertson DG. Comparative genomic hybridization. Annu Rev Genomics Hum Genet. 2005;6(1):331-54. **http://dx.doi.org/10.1146/annurev.genom.6.080604.162140**. PMid:16124865.
58. Su M-T, Lin S-H, Chen Y-C, Kuo P-L. Gene-gene interactions and gene polymorphisms of VEGFA and EG-VEGF gene systems in recurrent pregnancy loss. J Assist Reprod Genet. 2014;31(6):699-705. **http://dx.doi.org/10.1007/s10815-014-0223-2**. PMid:24671265.
59. Rajcan-Separovic E, Qiao Y, Tyson C, Harvard C, Fawcett C, Kalousek D, et al. Genomic changes detected by array CGH in human embryos with developmental defects. Mol Hum Reprod. 2010;16(2):125-34. **http://dx.doi.org/10.1093/molehr/gap083**. PMid:19778950.
60. Teng FYH, Wang Y, Tang BL. The syntaxins. Genome Biol. 2001;2(11):S3012. **http://dx.doi.org/10.1186/gb-2001-2-11-reviews3012**. PMid:11737951.
61. Zhang Y, Shu L, Chen X. Syntaxin 6, a regulator of the protein trafficking machinery and a target of the p53 family, is required for cell adhesion and survival. J Biol Chem. 2008;283(45):30689-98. **http://dx.doi.org/10.1074/jbc.M801711200**. PMid:18779328.
62. Higginbotham H, Bielas S, Tanaka T, Gleeson JG. Transgenic mouse line with green-fluorescent protein-labeled centrin 2 allows visualization of the centrosome in living cells. Transgenic Res. 2004;13(2):155-64. **http://dx.doi.org/10.1023/B:TRAG.0000026071.41735.8e**. PMid:15198203.
63. Gil Villa AM, Cardona-Maya WD, Cadavid Jaramillo ÁP. Muerte embrionaria temprana: ¿Tiene influencia el factor masculino? Arch Esp Urol. 2007;60(9):057-068. **http://dx.doi.org/10.4321/S0004-06142007000900002**. PMid:18077859.
64. Rawe VY, Terada Y, Nakamura S, Chillik C, Olmedo S, Chemes H. A pathology of the sperm centriole responsible for defective sperm aster formation, syngamy and cleavage. Hum Reprod. 2002;17(9):2344-9. **http://dx.doi.org/10.1093/humrep/17.9.2344**. PMid:12202423.

***Correspondence**

Sheila Janaina Sestari
Federal University of Goias (UFG), Institute of Biological Sciences, Department of Genetics
Avenida Esperança, s/n, Campus Samambaia (Campus II)
Zip code 74690-900, Goiania, Goias, Brazil
+55 62 9929-2549
E-mail: sheilasestari@hotmail.com

Authors information

SJS – PhD in Biological Sciences (Genetics) from the Federal University of Goias (UFG). BFG – PhD in Genetics from the Universidade Estadual Paulista (UNESP). FPFF – Graduated from the Federal University of Goias (UFG). RMOFC – PhD in Health Sciences from Faculty of Medicine, Federal University of Goias (UFG). ECC – PhD in in Health Sciences from Faculty of Medicine, Federal University of Goias (UFG); Gynecologist and Obstetrician at Humana Medicina Reprodutiva; Professor at the Faculty of Medicine, Federal University of Goias (UFG). NAB - PhD in Biological Sciences (Genetics) from the Universidade Estadual Paulista (UNESP); Professor at the Federal University of Goias (UFG). LARB - PhD in Biological Sciences (Genetics) from the Universidade Estadual Paulista (UNESP); Professor at the Federal University of Goias (UFG).

Authors contribution

SJS was responsible for study design and conceptualization, screening studies for inclusion, data extraction and database searches, data analysis and interpretation, manuscript drafting, critical review and manuscript comments. BFG was responsible for study design and conceptualization, screening studies for inclusion, data extraction and database searches, data analysis and interpretation, critical review and manuscript comments. FPFF was responsible for screening studies for inclusion, data extraction and database searches. RMOFC was responsible for screening studies for inclusion, data extraction and database searches. ECC was responsible for critical review and manuscript comments. NAB was responsible for study design and conceptualization. LARB was responsible for study design and conceptualization, data extraction and database searches, data analysis and interpretation, manuscript drafting, critical review and manuscript comments.

Appendix A

Table A1. Search terms with corresponding Mesh terms.

PubMed, Scielo, LILACS and Portal de CAPES/MEC (Limited to Human)	
OR	§ Abortion, Spontaneous
	§ Pregnancy loss
	§ Miscarriage
	§ Miscarriage, Recurrent
AND	
OR	§ Copy number Variation
	§ CNV
	§ Couple
	§ Microarray analysis
	§ Comparative Genomic Hybridization
	§ Array CGH

CNV, copy number variation; CGH, comparative genomic hybridization array.

Table A2. Inclusion and exclusion criteria for studies included in the systematic review.

Include	- Studies that addressed the analysis of CNVs in couples and women with a history of 2 or more consecutive idiopathic miscarriages up to the 22nd week of pregnancy
	- Idiopathic RM couples only
Exclude	- Studies that addressed the analysis of CNVs in couples and women with 2 or more miscarriages after the 22nd week of pregnancy
	- Studies that addressed the analysis of CNVs in couples and women with only 1 pregnancy loss
	- Studies that addressed the analysis of CNVs in couples and women with 2 or more miscarriages up to the 22nd week of pregnancy, but with known etiology
	- Studies in couples with infertility

Table A3. Modified Newcastle-Ottawa Scale.

Criteria	Score
Sample size	
> 100	2
10 - 100	1
1 - 10	0
Inclusion-Exclusion	
Described in full	2
Limited Description	1
Not included	0
Genetic Methodology	
CGH array, SNP array or Sequencing	2
Other	1
Not mentioned	0
Statistical Analysis	
Well described statistics	1
No Statistics Mentioned	0
Case Definition	
Independent Validation	1
Self-reported	0
Controls	
Yes	1
No	0
Comparability	
Comparison of CNVs between the case-control group or with the DGV database	1
Not applicable/ no comparison	0

Table A4. Individual Scores for Newcastle-Ottawa Scale

Paper	Sample Size (0-2)	Inclusion/ Exclusion (0-2)	Genetic Methodology (0-2)	Statistical analysis (0-1)	Case Definition (0-1)	Controls (0-1)	Comparability (0-1)	Total (10)
Karim et al., 2017	1	2	2	1	1	0	1	8
Kasak et al., 2017	1	2	2	1	1	1	1	9
Nagirnaja et al., 2014	2	2	2	1	1	1	1	10
Rajcan-Separovic et al., 2009	1	2	2	0	0	1	1	7
Rajcan-Separovic et al., 2010	1	2	2	0	1	1	1	8