

Potential genetic causes of miscarriage in euploid pregnancies

Morgan, Neil; Colley, Emily; Smith, Paul; Allen, Stephanie; Hamilton, Susan; Coomarasamy, Arri

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1 **Potential genetic causes of miscarriage in euploid pregnancies: A**
2 **systematic review**

3

4 **Running title:** Genetic causes of miscarriage in euploid pregnancies

5

6 **Authors:** Emily Colley^{1, 2}, Susan Hamilton^{1, 3}, Paul Smith^{1, 2}, Neil V. Morgan², Arri
7 Coomarasamy^{1,2} and Stephanie Allen^{1,3}

8 ¹Tommy's National Centre for Miscarriage Research, Birmingham Women's and Children's
9 Hospital, Birmingham, B15 2TG

10 ²The University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom

11 ³West Midlands Regional Genetics Laboratory, Birmingham Women's and Children's
12 Hospital, Birmingham B15 2TG

13

14 **Corresponding author:** Emily Colley

15 **Corresponding author ORCID iD:** orcid.org/0000-0001-6373-750X

16 **Corresponding author e-mail:** ECC669@bham.ac.uk

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48 **Abstract**

49 **BACKGROUND:** Approximately 50% of pregnancy losses are caused by chromosomal
50 abnormalities, such as aneuploidy. The remainder have an apparent euploid karyotype, but
51 it is plausible that there are cases of pregnancy loss with other genetic aberrations that are
52 not currently routinely detected. Studies investigating the use of exome sequencing and
53 chromosomal microarrays in structurally abnormal pregnancies and developmental
54 disorders have demonstrated their clinical application and/ or potential utility in these
55 groups of patients. Similarly, there have been several studies that have sought to identify
56 genes that are potentially causative of, or associated with, spontaneous pregnancy loss, but
57 the evidence has not yet been synthesized.

58

59 **OBJECTIVE AND RATIONALE:** The objective was to identify studies which have recorded
60 monogenic genetic contributions to pregnancy loss in euploid pregnancies, establish
61 evidence for genetic causes of pregnancy loss, identify the limitations of current evidence
62 and make recommendations for future studies. This evidence is important in considering
63 additional research into Mendelian causes of pregnancy loss and appropriate genetic
64 investigations for couples experiencing recurrent pregnancy loss.

65

66 **SEARCH METHODS:** A systematic review was conducted in MEDLINE (1946 to May 2018)
67 and Embase (1974 to May 2018). The search terms “spontaneous abortion”, “miscarriage”,
68 “pregnancy loss” or “lethal” were used to identify pregnancy loss terms. These were
69 combined with search terms to identify the genetic contribution including “exome”, “human
70 genome”, “sequencing analysis”, “sequencing”, “copy number variation”, “single nucleotide
71 polymorphism”, “microarray analysis” and “comparative genomic hybridization”. Studies

72 were limited to pregnancy loss up to 20 weeks in humans, and excluded if the genetic
73 content included genes which are not lethal *in utero*, PGD studies, infertility studies,
74 expression studies, aneuploidy with no recurrence risk, methodologies where there is no
75 clinical relevance and complex genetic studies. The quality of the studies was assessed using
76 a modified version of the Newcastle-Ottawa scale.

77

78 **OUTCOMES:** A total of 50 studies were identified and categorized into three themes; whole
79 exome sequencing studies, copy number variation studies and other studies related to
80 pregnancy loss including recurrent molar pregnancies, epigenetics and mitochondrial DNA
81 aberrations. Putatively causative variants were found in a range of genes, including
82 [cholinergic receptor, nicotinic, alpha polypeptide 1 \(CHRNA1\)](#), [dynein, cytoplasmic 2, heavy](#)
83 [chain 1 \(DYNC2H1\)](#) and [ryanodine receptor 1 \(RYR1\)](#), which were identified in multiple
84 studies. Copy number variants were also identified to have a causal or associated link with
85 recurrent miscarriage.

86

87 **WIDER IMPLICATIONS:** Identification of genes that are causative of or predisposing to
88 pregnancy loss will be of significant individual patient impact with respect to counselling and
89 treatment. In addition, knowledge of specific genes that contribute to pregnancy loss could
90 also be of importance in designing a diagnostic sequencing panel for patients with recurrent
91 pregnancy loss, and also in understanding the biological pathways that can cause pregnancy
92 loss.

93

94 **Key words:** genetic causes, pregnancy loss, euploid miscarriage, exome sequencing,
95 chromosomal array, single nucleotide variation, copy number variant.

96

97

98 **Introduction**

99 AUTHOR: I suggest that a short introductory paragraph here, placing the study in context,
100 would be helpful to the reader. Please would you add a sentence or two to achieve this?

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101 Miscarriage and recurrent pregnancy loss

102 Approximately 15-% of clinically recognised pregnancies end in pregnancy loss, with the
103 majority occurring during the first trimester. Of these, 50-% are caused by chromosomal
104 abnormalities such as aneuploidy (Hassold et al., 1980), and can be detected by
105 conventional cytogenetic analysis. It is suggested that 86 % of these abnormalities are
106 numerical, 6 % are structural abnormalities and 8 % are due to other genetic mechanisms,
107 such as chromosomal mosaicism and molar pregnancies (Goddijn and Leschot, 2000).

108

109 Recurrent Miscarriage (RM) is defined by the Royal College of Obstetricians and
110 Gynaecologists (RCOG) as at least three consecutive miscarriages before 24 weeks gestation
111 (RCOG, 2011) and recurrent pregnancy loss (RPL) by the ESHRE November 2017 guidelines
112 as the loss of two or more pregnancies (ESHRE, 2017). In addition to genetic aetiology, a
113 spectrum of non-genetic causes of RPL have also been identified, including thrombophilic
114 factors, endocrinological causes, immunological and immunogenetic causes, sperm DNA
115 fragmentation, uterine malformations and lifestyle factors such as smoking (reviewed by

116 **Larsen et al. 2013).**

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117

118 Cytogenetic and chromosomal microarray analysis

119 Traditionally, cytogenetic analysis of pregnancy tissue has been performed to identify
120 genetic causes of RPL, and to indicate the need for further analysis of parental samples
121 where there is the possibility of a balanced chromosome rearrangement (e.g. translocation)
122 in one of the parents. It is important to identify any numeric chromosome errors, such as
123 trisomy, monosomy or polyploidy, since these are causes of pregnancy loss which usually
124 occur sporadically, and the likelihood of a successful pregnancy outcome is not negatively
125 affected in subsequent pregnancies. Where there is a balanced translocation in one of the
126 parents, genetic counselling is important as there is likely to be a recurrence risk in future
127 pregnancies and pre-implantation genetic testing, chorionic villus sampling or amniocentesis
128 can be used to detect an abnormality in the conceptus. However, for couples with a
129 translocation, medical management (e.g. natural conception and observation) has been
130 reviewed to have a higher live birth rate than IVF/PGD (Franssen et al., 2011, Hirshfeld-
131 Cytron et al., 2011).

132
133 The most recent ESHRE guidelines for genetic analysis of products of conception (POC) give
134 a conditional recommendation for genetic analysis but recommend that testing is carried
135 out by array-comparative genomic hybridization (CGH) instead of traditional karyotyping
136 (ESHRE, 2017). Conventional karyotype analysis identifies balanced and unbalanced
137 chromosomal rearrangements and copy number variants (CNVs) to an approximately 5Mb
138 resolution. Chromosomal microarray analysis can now identify unbalanced CNVs below
139 1Mb, with a resolution at the level of individual exons of genes in targeted regions of the
140 genome (Miller et al., 2010). Microarray analysis is also less labour intensive as it is based on
141 DNA analysis rather than cultured cells and has a higher success rate in poor quality tissue
142 samples, however the quality of tissue will impact the success and failure rate of both

143 conventional karyotyping and array-CGH. Array-CGH has become the gold standard for
144 genetic CNV analysis. It should, however, be noted that array-CGH may miss some balanced
145 chromosomal rearrangements and may also fail to identify maternal cell contamination.

146

147 Other genetic causes

148 In the case of pregnancy loss, with an apparently euploid karyotype, there may be genetic
149 aberrations causative of pregnancy loss that are not currently known or routinely assessed.

150 These could include single-nucleotide variants (SNVs) that affect individual genes and are
151 detectable by sequencing or small sub-microscopic aberrations that affect a cluster of genes
152 and are detectable by microarray analysis. In the case of SNVs this is particularly important
153 as many may follow a recessive or X-linked pattern of inheritance and therefore have a high
154 recurrence risk. CNVs detected in cases of pregnancy loss may unmask a recessive mutation
155 in a relevant gene or involve dosage sensitive genes, where loss or gain of copies affects the
156 gene function. These regions may also represent benign CNVs seen frequently with no
157 recorded effect on phenotype, although it remains possible that some may be involved in
158 RPL. Evidence in humans and other species (Wilson et al., 2016) suggests that many genes
159 are important in early development, and can lead to embryonic lethality when functionally
160 “knocked out”, resulting in pregnancy loss. More widespread genetic analysis of embryonic
161 pregnancy loss may provide an opportunity to identify genes that are essential in early
162 human development or where a lack of function leads to pregnancy loss.

163

164 Molar pregnancies

165 A molar pregnancy or Hydatidiform mole (HM) is an abnormal pregnancy, which has cystic
166 degeneration of the chorionic villi, abnormal proliferation of the trophoblast and abnormal

167 | development of the fetus. These can either be complete HM (~~CHM~~) or partial HM,
168 | distinguishable by the extent of trophoblast proliferation and presence of embryonic tissue.
169 | CHMs are usually diploid with all chromosomes of paternal origin. The majority arise from
170 | an anuclear ovum being fertilised by a haploid sperm and replicating its own chromosomes
171 | (uniparental paternal isodisomy), or rarely from an anuclear ovum fertilised by two sperm
172 | (uniparental paternal heterodisomy). HMs are mostly triploid with 23 chromosomes of
173 | maternal origin and 46 of paternal origin.

174

175 | Whilst HMs are usually triploid and sporadic and therefore outside the scope of this review,
176 | a minority of molar pregnancies are diploid and biparental, usually being recurrent and
177 | familial. These may be caused by maternal autosomal recessive mutations in genes, such as
178 | [NLR family, pyrin domain-containing 7 \(NLRP7\)](#) and [KHDC3-like protein, subcortical maternal](#)
179 | [complex member \(KHDC3L\)](#), resulting in an abnormal epigenotype of imprinted loci. This
180 | results in abnormal gene expression, which causes abnormal placental trophoblast
181 | development and manifests as HM (Carey et al., 2015).

182

183 | Whole exome sequencing

184 | Advances in sequencing technology, including whole exome sequencing (WES) and whole
185 | genome sequencing (WGS), are increasingly providing the opportunity to detect genetic
186 | sequence variation and to characterise genetic mutations causing disease. WGS is the most
187 | extensive sequencing method and targets the entire genome, whereas WES targets the
188 | exome, which is the protein-coding region of the DNA. The exome makes up approximately
189 | 1% of the human genome, and it is estimated to contain 85% of the genetic mutations
190 | associated with disease (Choi et al., 2009). Generally, WES is the preferred method of

191 sequencing because it is cheaper than WGS and has a smaller, more manageable data set
192 whilst still comprehensively covering the coding regions of DNA. WGS has the advantage of
193 analysing and giving a comprehensive view of the whole genome and has the potential to
194 detect large structural variants, insertions/ deletions, SNVs and copy number changes.
195 However, we still understand relatively little about the non-coding regions of the genome.

196

197 Studies investigating the use of WES in structurally abnormal pregnancies, late pregnancy
198 losses and developmental disorders (Wright et al., 2015, Shamseldin et al., 2018, Carss et
199 al., 2014) have demonstrated the clinical application in these patients. However, very few
200 WES studies have reported analysis in pregnancy loss or lethal genes which could contribute
201 to RPL. The few studies using WES to look for genetic aberrations in RPL have also tended to
202 represent only small patient cohorts. The ability to recognise and detect genetic mutations
203 may have implications for routine genetic testing and clinical practice, especially when a
204 pathogenic aberration is identified that can be reliably detected in future pregnancies.

205

206 Aims

207 There are several studies that have sought to identify genes causative of or associated with
208 pregnancy loss, but the evidence has not yet been synthesised. We propose to review these
209 studies and establish evidence of genetic causality of RPL, including reviewing appropriate
210 methodologies. We will evaluate studies investigating Mendelian inheritance patterns,
211 including autosomal recessive and dominant X-linked inheritance, and also *de novo* genetic
212 causes, but we have excluded studies investigating more complex genetic associations,
213 which have recently been systematically reviewed (Pereza et al., 2017).

214

215 **Methods**

216 Registration

217 This systematic review has been registered with PROSPERO (CRD42017073910).

218

219 Search

220 A systematic literature review to assess the studies investigating the genetic contribution to
221 RPL was conducted in MEDLINE (1946 to May 2018) and Embase (1974 to May 2018) using
222 Ovid (<https://ovidsp.tx.ovid.com>). The search terms used to identify pregnancy loss were
223 “Spontaneous abortion”, “miscarriage”, “pregnancy loss” or “lethal”, and the search terms
224 to identify the genetic contributions are “exome”, “human genome”, “sequencing analysis”,
225 “sequencing”, “copy number variation”, “single nucleotide polymorphism”, “microarray
226 analysis” and “comparative genomic hybridisation”. The search terms and corresponding
227 Mesh terms are shown in Supplementary Table S1. Additional studies were also identified
228 from references of selected studies.

229

230 Study selection

231 Studies were selected by two independent reviewers. Studies were first screened for
232 eligibility using article titles and then by screening the study abstracts. Studies were
233 included if they had pregnancy loss up to 20 weeks, but were not restricted if they also
234 included some later losses, providing the genetic aberrations were defined. Studies were
235 excluded if the genetic content included genes which were not lethal *in utero*, PGD studies,
236 infertility studies, expression studies, aneuploidy with no recurrence risk, methodologies
237 where there is no clinical relevance, and complex genetics. Both recurrent and sporadic

238 pregnancy loss were included. The full inclusion and exclusion criteria are presented in
239 Supplementary Table SII.

240

241 Data extraction process

242 Data on publication date, country, study objective, sample, phenotype and gestation,
243 methods and analysis, study outcome and quality scores were extracted. Data extraction
244 was checked by a second reviewer. Each of the identified genes were found in Online
245 Mendelian Inheritance in Man (OMIM) and the Mendelian Inheritance in Man (MIM)
246 number, Gene name, gene function, associated disease/phenotype and cytogenetic location
247 were ascertained.

248

249 Quality assessment

250 The quality of each study was assessed using a modified Newcastle-Ottawa scale
251 (Supplementary Table SIII). Each study was scored out of 12 and was judged on the sample
252 size, inclusion/exclusion criteria, the genetic analysis method, statistical analysis, case
253 definition, controls and comparability. The breakdown of each score is included in
254 Supplementary Table SIV.

255

256 **Results**

257 A total of 50 studies were included in the review. The initial search of the Medline and
258 Embase databases identified 3404 potentially relevant articles. After screening the titles and
259 abstracts, 74 full texts were obtained for detailed review. A total of 30 full articles were
260 excluded because they were either not related to pregnancy loss, were more than 20 weeks
261 gestation, or contained no genetic content. Examination of the bibliographies and journal

262 indices generated six additional studies for the review. Figure 1 illustrates the study
263 selection. The papers identified were categorized into three themes; WES studies, CNV
264 studies and other studies related to pregnancy loss including recurrent molar pregnancies.

265

266 The 50 studies that met the inclusion and exclusion criteria were all published in English
267 between 2009 and 2018. Out of the studies identified, 21 were from Europe, 14 were from
268 North America, 13 were from Asia and there was one study each from South America and
269 Africa.

270

271 WES

272 Thirteen studies were identified (Table I) which used WES to identify SNVs in families with
273 multiple pregnancy losses or a combination of pregnancy losses and terminations. Eight of
274 these studies focused on a single couple only (Bondeson et al., 2017, Cristofoli et al., 2017,
275 Dohrn et al., 2015, Filges et al., 2014, Rae et al., 2015, Shamseldin et al., 2013, Tsurusaki et
276 al., 2014, Wilbe et al., 2015). Six studies used WES analysis of trios (Filges et al., 2014, Dohrn
277 et al., 2015, Wilbe et al., 2015, Cristofoli et al., 2017, Bondeson et al., 2017, Qiao et al.,
278 2016).

279

280 Studies using WES identified variants in genes from both fetal and parental samples, thus
281 allowing for the inheritance to be identified. One study identified compound heterozygous
282 mutations in [kinesin family member 14 \(KIF14\)](#) in a family with unexplained euploid
283 miscarriages (Filges et al., 2014). The other studies included pregnancies terminated for a
284 fetal abnormality including; a homozygous missense mutation in [endothelin-converting](#)
285 [enzyme-like 1 \(ECE1\)](#) from a consanguineous couple with pregnancies terminated due to

286 Arthrogyrosis Multiplex Congenita (Dohrn et al., 2015); a novel homozygous mutation in
287 the [muscle, skeletal, receptor tyrosine kinase \(*MuSK*\)](#) gene in a non-consanguineous couple
288 with a history of fetal akinesia deformation sequence (FADS) (Wilbe et al., 2015); compound
289 heterozygous mutations in [SCL/TAL1-interrupting locus \(*STIL*\)](#) from a non-consanguineous
290 couple with fetal microcephaly (Cristofoli et al., 2017), a homozygous nonsense mutation in
291 [centrosomal protein, 55-KD \(*CEP55*\)](#) in a non-consanguineous family with [two2](#) fetuses with
292 Meckel-like syndrome (Bondeson et al., 2017) and compound heterozygous mutations in
293 [intraflagellar transport 122 \(*IFT122*\)](#) in a couple experiencing both RPL and later losses with
294 scan abnormalities (Tsurusaki et al., 2014).

295

296 Two studies (Rae et al., 2015, Shamseldin et al., 2013) identified pathogenic variants by WES
297 of fetuses affected with hydrops fetalis. The first identified pathogenic variant in the gene
298 [forkhead box P3 \(*FOXP3*\)](#) was from a non-consanguineous couple whom had multiple male
299 pregnancy terminations. *FOXP3* is an X-linked gene which is known to cause fetal akinesia
300 syndrome (Rae et al., 2015). The second identified novel mutation in the gene [cholinergic
301 receptor, nicotinic, alpha polypeptide 1 \(*CHRNA1*\)](#) was identified in a consanguineous couple
302 (Shamseldin et al., 2013). Autosomal recessive mutations in this gene are also known to
303 cause fetal akinesia.

304

305 A single study identified a homozygous missense variant in [nucleolar protein 14 \(*NOP14*\)](#) in
306 pregnancy loss material from two consanguineous Iranian couples experiencing RPL. WES
307 was completed on fetal tissue samples and the heterozygous copies of the variant were
308 confirmed in the parents using Sanger sequencing (Suzuki et al., 2018).

309

310 Studies also used WES in larger cohorts. One study (Shamseldin et al., 2015) looked at
311 consanguineous couples with two or more pregnancies diagnosed with non-immune
312 hydrops fetalis (NIHF). Seven pathogenic variants previously known to cause NIHF
313 (Shamseldin et al., 2015) were identified from 24 consanguineous couples with lethal NIHF.

314

315 Two Studies (Ellard et al., 2015, Qiao et al., 2016), analysed non-consanguineous couples
316 with RPL. Variants in [RNA export mediator \(GLE1\)](#), [ryanodine receptor 1 \(RYR1\)](#) and [DYNEIN,](#)
317 [cytoplasmic 2, heavy chain 1 \(DYNC2H1\)](#) were identified using WES of parental samples only
318 (Ellard et al., 2015). Compound heterozygous variants were also identified in *DYNC2H1* and
319 [15-lipoxygenase, reticulocyte arachidonate \(ALOX15\)](#) in seven euploid pregnancy losses
320 from four families (Qiao et al., 2016).

321

322 The final study used a slightly different approach and analysed a panel of 234 pre-selected
323 RPL candidate genes from women affected by RPL. Using WES and bioinformatic filtering of
324 non-synonymous sequence variants, 27 variants were identified from the previously
325 selected genes (Quintero-Ronderos et al., 2017). The genes in which variants were identified
326 in the described sequencing studies are detailed in Table II. However, genes from Quintero-
327 Ronderos et al. [2017](#) have been excluded because they were from a pre-selected gene panel
328 and therefore would introduce bias.

329

330 CNVs

331 Thirteen studies and one meta-analysis (Bagheri et al., 2015) (Table III), were identified
332 which looked for CNVs in fetal tissue, parental samples or both by chromosomal microarray
333 analysis. Three different microarray platforms were used for analysis, either single
334 nucleotide polymorphism (SNP) array, oligonucleotide (oligo) array or bacterial artificial
335 chromosome (BAC) array.

336

337 Six studies reported CNVs in pregnancy loss (Zhang et al., 2009, Viaggi et al., 2013, Levy et
338 al., 2014, Zhang et al., 2016, Donaghue et al., 2017, Zhou et al., 2016), four studies in RPL
339 (Rajcan-Separovic et al., 2010a, Nagirnaja et al., 2014, Karim et al., 2017, Robberecht et al.,
340 2012) and three studies with a mixture of both pregnancy loss and RPL (Wang et al., 2017,
341 Warren et al., 2009, Rajcan-Separovic et al., 2010b). Seven of the studies included parental
342 samples and therefore the inheritance of reported CNVs was determined. Six of the studies
343 did not include parental samples, and therefore the inheritance pattern of the CNVs
344 reported in these studies could not be determined.

345

346 The pregnancy losses reported were pregnancies of varying gestational age, with the
347 majority of pregnancy losses at less than 20 weeks. In three studies (Rajcan-Separovic et al.,
348 2010a, Robberecht et al., 2012, Viaggi et al., 2013), all pregnancy losses tested were less
349 than 12 weeks gestation. Two papers (Rajcan-Separovic et al., 2010b, Robberecht et al.,
350 2012) also identified pregnancies with developmental abnormalities and used hystero-
351 embryoscopy to allow morphological examination of the fetus *in utero* prior to genetic
352 analysis.

353

354 Of the studies which determined the inheritance of the CNVs, there were 30 *de novo*, and
355 43 inherited CNVs (Levy et al., 2014, Rajcan-Separovic et al., 2010a, Rajcan-Separovic et al.,
356 2010b, Robberecht et al., 2012, Wang et al., 2017, Warren et al., 2009). In general, the
357 studies showed a 2.2 % - 13 % detection rate (DR) of pathogenic CNVs (Donaghue et al.,
358 2017, Levy et al., 2014, Wang et al., 2017, Warren et al., 2009, Zhang et al., 2016, Zhang et
359 al., 2009) plus a 0.9 % to 3.3 % DR of variants of unknown significance (VOUS) (Donaghue et
360 al., 2017, Wang et al., 2017, Zhang et al., 2016, Qiao et al., 2016). An additional meta-
361 analysis study (Bagheri et al., 2015) compared the characteristics and contributions of rare
362 and common CNVs from four of the other studies by reclassifying CNVs according to the
363 prevalence of healthy controls using Database of Genomic Variants (Bagheri et al., 2015,
364 Rajcan-Separovic et al., 2010a, Rajcan-Separovic et al., 2010b, Robberecht et al., 2012,
365 Viaggi et al., 2013). They concluded that common CNVs were specifically enriched in
366 immunological pathways and rare CNVs were not, although the small number of rare CNVs
367 may have hampered this conclusion. However, both rare and common CNVs could have a
368 role in pregnancy loss, as rare CNVs have a two times higher gene density and contain more
369 genes studied in mouse knockouts and common CNVs contain more genes in biological
370 pathways relevant to pregnancy. The studies which identified VOUS were in accordance
371 with each other and suggested the rate of 2-3 %.

372

373 Of particular interest is to find recurrent CNVs that are associated with pregnancy loss.
374 Maisenbacher et al (Maisenbacher et al., 2017) determined the frequency of the 22q11.2
375 deletion in a large cohort of pregnancy loss samples using a SNP microarray. The 22q11.2
376 deletion was detected in 15 (0.07%) of 22451 POCs, with an overall incidence of 1/-1497.

377 They concluded that this was higher than the reported general population prevalence
378 (1/4000- 1/6000). Likewise, Nagirnaja et al. (2014) identified CNV regions on chromosome 5
379 (5p13.3), disrupting the [PDZ domain-containing 2 \(PDZD2\)](#) and [golgi phosphoprotein 3](#)
380 [\(GOLPH3\)](#) genes. There was significant association with an increased risk of RPL. *PDZD2* and
381 *GOLPH3* are predominately expressed in the placenta, suggesting a functional relevance,
382 however neither of these genes has previously been linked to placental function or
383 pregnancy complications (Nagirnaja et al., 2014).

384

385 Recurrent molar pregnancies

386 Eleven studies (Table IV) were identified which evaluated the genetics of diploid and
387 biparental recurrent HM (RHM) pregnancies. One study (Parry et al., 2011) identified
388 biallelic mutations in [chromosome 6 open reading frame 221 \(C6orf221\)](#) in three
389 consanguineous families with familial biparental HM. Three studies (Abdalla et al., 2012,
390 Brown et al., 2013, Ulker et al., 2013) reported case studies of an individual consanguineous
391 family, two non-consanguineous families and two consanguineous families with RHM.
392 Autosomal recessive mutations were identified in the *NLRP7* gene and were considered to
393 be responsible for the occurrence of HM. Deveault et al. investigated 13 women
394 experiencing RHM, some with a family history of molar pregnancies and 11 *NLRP7* variants
395 were identified (Deveault et al., 2009). Mutation analysis of the *NLRP7* gene in 35 women
396 experiencing RPL with at least one HM revealed 17 different mutations (Qian et al., 2011).
397 Qian et al. (2011) also suggested that one defective allele in *NLRP7* causes diploid
398 androgenic moles and two defective alleles causes diploid biparental moles.

399

400 Two studies (Huang et al., 2013, Messaed et al., 2011) investigated cohorts of women to see
401 whether mutations in the *NLRP7* gene could also be responsible for RPL without history of
402 molar pregnancy. Messaed et al. (2011) investigated 135 women with either RPL or at least
403 one HM and sequencing of *NLRP7* exons identified two patients with RPL to have *NLRP7*
404 mutations. Huang et al. (2013) also showed significant association between RPL and *NLRP7*
405 polymorphisms. In contrast, two further studies (Andreasen et al., 2013, Manokhina et al.,
406 2013) identified no disease-causing mutations in *NLRP7* in women with RPL and similarly
407 Aghajanova et al. (Aghajanova et al., 2015) found no mutations in *NLRP7*, [NLR family, pyrin](#)
408 [domain-containing 2 \(*NLRP2*\)](#) or [KHDC3-like protein, subcortical maternal complex member](#)
409 [\(*KHDC3L*\) \(*C6orf221*\)](#).

410

411 Other genetic causes

412 Two studies (Seyedhassani et al., 2010a, Seyedhassani et al., 2010b) analysed and
413 sequenced mitochondrial tDNA (**AUTHOR: is tDNA correct here?**) in 96 women with RPL.
414 Four variants in threonine transfer RNA (tRNA) and one variant in proline tRNA were
415 observed, but in some cases these were also observed in controls (Seyedhassani et al.,
416 2010a), which calls into question the significance of these findings. Analysis of mitochondrial
417 D-loop sequences showed a higher rate of point mutations in RPL patients than in controls.
418 In total, 89 out of 153 variants were only identified in women with RPL and 22 of these
419 mutations were considered to be significant (Seyedhassani et al., 2010b).

420

421 X-chromosome inactivation occurs during early embryogenesis and has also been proposed
422 to have an aetiological role in RPL. Skewed X-chromosome inactivation (XCI) status was
423 compared between women with RPL and healthy controls. Extremely skewed XCI (defined

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424 as >90 %) was identified in 17.7% of women with RPL compared to 1.6 % of extremely
425 skewed XCI in controls (Bagislar et al., 2006).

426

427 Six further papers were identified that discussed specific genes and their contribution to
428 pregnancy loss. Each paper (Bendroth-Asmussen et al., 2016, Bhuiyan et al., 2008, Lopez-
429 Carrasco et al., 2013, McKie et al., 2014, Stouffs et al., 2011, Zhang et al., 2016) investigated
430 an individual gene or genes. In a case study of a 30-year-old women with pregnancy loss
431 from glycogen storage disease Type IV (GSD-IV), DNA extracted from placental tissue
432 identified compound heterozygous mutations in [glycogen branching enzyme \(GBE1\)](#)
433 (Bendroth-Asmussen et al., 2016).

434 Another case study, a consanguineous Arabian family with pregnancy losses, stillborn, fetal
435 demise and two live children, had homozygosity mapping. This led to the screening of the
436 [human ether-a-go-go-related gene \(HERG\)](#) gene in the live children, parents and stillborn.
437 Homozygous nonsense mutations in *HERG* were identified in the child with polymorphic
438 ventricular tachycardia and the same heterozygous mutation in the parents and unaffected
439 child. Amniotic fluid cells from the stillborn child were also homozygous for the same *HERG*
440 mutation (Bhuiyan et al., 2008).

441

442 Three rare homozygous [RYANODINE RECEPTOR 1 \(RYR1\)](#) variants were identified using
443 genome-wide linkage studies and sequencing of *RYR1* coding exons. Initially a *RYR1*
444 homozygous nonsense mutation was detected in two fetuses with fetal akinesia
445 deformation sequence (FADS)/ lethal multiple pterygium syndrome (LMPS). The parents
446 were both homozygous for the same mutation. When 66 further probands with FADS/ LMPS

447 phenotype were screened for germline *RYR1* mutations, two further potential homozygous
448 mutations were detected (McKie et al., 2014).

449

450 In a larger study, 100 couples with at least three unexplained pregnancy losses had
451 [wingless-type MMTV integration site family, member 6 \(*WNT6*\)](#) mutation analysis
452 performed. *WNT6* has previously been shown to have an important role for stromal cell
453 proliferation during decidualisation in mice. Four novel mutations were identified in the
454 women with RPL but not in the male partners or healthy controls (Zhang et al., 2015),
455 although there was no conclusive evidence for pathogenicity.

456

457 Ten aberrations were identified in [MutS, E. coli, homolog of, 4 \(*MSH4*\)](#), [DNA](#)
458 [methyltransferase 3-like protein \(*DNMT3L*\)](#) and [synaptonemal complex protein 3 \(*SYCP3*\)](#) in
459 23 couples with RPL. Six of these aberrations were predicted to alter the amino acid
460 sequence. All but one of these aberrations was considered a likely SNV. The mutation in the
461 *SYCP3* gene was shown to have a 78 % likelihood of causing a deleterious effect on protein
462 function due to an alteration in the amino acid sequence changing a non-polar isoleucine
463 into a polar threonine (Stouffs et al., 2011). Another study (Lopez-Carrasco et al., 2013)
464 targeted the two spindle checkpoint genes [aurora kinase B \(*AURKB*\)](#) and *SYCP3* in 102
465 patients with either RPL or spermiogram alterations. One heterozygous intronic deletion
466 was identified in *SYCP3* with no *in silico* causative indication. Six aberrations were identified
467 in *AURKB*, however a deletion and two nucleotide changes were considered to have no
468 functional alteration or be frequent variants respectively. Three rare missense variants were
469 identified in *AURKB*, with two of these variants found in a couple with pregnancy loss.

470

471 **Discussion**

472 In this systematic review we have identified 50 papers which investigated genetic
473 contributions other than aneuploidy to pregnancy loss. The studies highlight some key
474 areas, including identification of SNVs by WES, identification of CNVs by microarray analysis,
475 and investigation of a group of genes associated with diploid and biparental recurrent molar
476 pregnancies that are linked to pregnancy loss. Other genetic contributions, such as
477 epigenetics and mitochondrial DNA (mtDNA), were also investigated in individual papers.
478 There were also studies reporting sequencing of candidate genes already known to be
479 associated with pregnancy loss with or without structural abnormalities.

480

481 We have summarised the current evidence below for each of these categories, and then
482 discuss the implications of these findings both for future studies and for genetic
483 investigation of couples experiencing RPL.

484

485 WES

486 Advances in next generation sequencing are vastly improving and enabling a molecular
487 diagnosis for a range of disorders and clinical pathways. As the cost of WES decreases, the
488 technology is becoming more widely used and clinically applicable. This review identified a
489 number of studies (Table I) over the last 4 years which have used WES to look for as yet
490 unidentified genetic causes of pregnancy loss. The majority of these studies looked at
491 individual patients or couples with RPL, some of which showed ultrasound scan
492 abnormalities during the pregnancy (Bondeson et al., 2017, Cristofoli et al., 2017, Wilbe et
493 al., 2015, Tsurusaki et al., 2014). More recently a small number of studies have been
494 published studying larger cohorts of patients and exploring possible strategies for genetic

495 investigation of these patients (Ellard et al., 2015, Qiao et al., 2016, Shamseldin et al., 2015).
496 This review included studies where patients suffered multiple pregnancy losses with
497 phenotypic findings in all or some of their pregnancy losses. This included ultrasound scan
498 abnormalities and post-mortem findings, and in some cases, where patients opted for
499 termination of pregnancy. These were thought to be important to include because there
500 could be a range of phenotypic effects caused by a genetic abnormality in a lethal gene,
501 which could include abnormalities and late fetal death in some pregnancies, but pregnancy
502 loss in others.

503
504 Bioinformatic filtering is required when studying the whole exome in order to provide a
505 more manageable approach to interpretation of the data. In most of these studies 'trios' of
506 patients were sequenced, and bioinformatic modelling of inheritance patterns was used to
507 limit the number of variants identified. In most cases patterns of autosomal recessive
508 inheritance (or X-linked recessive in male fetal losses) were modelled to look for variants.
509 As might be expected, very often the couples investigated were consanguineous or possibly
510 from populations isolated geographically. An alternative autozygosity mapping approach
511 was used by Shamseldin et al. to restrict the genes that were analysed by WES (Shamseldin
512 et al., 2013, Shamseldin et al., 2015) and a 'proof of principle' study (Ellard et al., 2015)
513 developed a technique to identify autosomal recessive lethal disorders using WES in couples
514 with RPL.

515
516 It is important to note that where autosomal recessive mutations are identified as a cause of
517 pregnancy loss, this will guide counselling and treatment options for the couple as there is a

518 1:4 recurrence risk in future pregnancies, and prenatal diagnosis or PGD would be available
519 to the couple.

520

521 Interestingly, genes that were identified from these WES studies are associated with
522 processes that have an early role in developmental biology and are essential in
523 embryogenesis. Some key processes include centrosome integrity, anti-inflammatory/
524 immune responses, proliferation and maintenance of epithelial cells, maintenance and
525 development of collagen and muscle tissues, and blood coagulation. The majority of WES
526 studies focused on individual families. Therefore the genes detected are limited to
527 preselected cases and it is not possible to group them together for a meta-analysis to
528 ascertain the detection rates.

529

530 Immune cells present early during pregnancy, especially during implantation where the
531 maternal immune system has to tolerate the implanting embryo. The immune response
532 during implantation is not currently well understood. However, the maternal immunity
533 shifts from cell-mediated immunity to humoral (antibody mediated) immunity to protect
534 the embryo from rejection. Aberrations in several genes, *ALOX15* (Qiao et al., 2016),
535 [complement component receptor 1 \(CR1\)](#) (Quintero-Ronderos et al., 2017), *FOXP3* (Rae et
536 al., 2015) and [TOLL-LIKE RECEPTOR 3 \(TLR3\)](#) (Filges et al., 2014) were identified and are
537 known to be involved in inflammatory and immune defences. Mutations in these genes
538 could be causing defects resulting in early pregnancy loss because the immune response is
539 rejecting the embryo.

540

541 During embryogenesis, cells differentiate and proliferate. Potentially causative mutations
542 were identified in [FMS-related tyrosine kinase 1 \(FLT1\)](#) (Quintero-Ronderos et al., 2017),
543 [leukemia inhibitory factor receptor \(LIFR\)](#) (Quintero-Ronderos et al., 2017) and [ubiquitin 1](#)
544 [\(UBN1\)](#) (Shamseldin et al., 2015) genes involved in cell differentiation and proliferation.
545 Mutations in the two genes [trophinin \(TRO\)](#) and [cadherin 11 \(CHD11\)](#) were both identified
546 (Quintero-Ronderos et al., 2017) and are involved in cell adhesion. As cell differentiation,
547 cell proliferation and cell adhesion are an important part of fetal growth during pregnancy,
548 disruption in these genes could cause the pregnancy to fail.

549
550 Mutations in genes involved in tissue formation were also identified. In particular, [cadherin](#)
551 [1 \(CDH1\)](#) (Quintero-Ronderos et al., 2017) and [frizzled, drosophila, homolog of, 6 \(FZD6\)](#)
552 (Shamseldin et al., 2015) are specifically involved in cell adhesion, [matrix metalloproteinase](#)
553 [10 \(MMP10\)](#) and [matrix metalloproteinase 9 \(MMP9\)](#) (Quintero-Ronderos et al., 2017) for
554 extracellular remodelling, and *MuSK* (Wilbe et al., 2015) and [myomesin 1 \(MYOM1\)](#)
555 (Shamseldin et al., 2015) for formation of neuromuscular junctions and striated muscle.

556
557 During pregnancy, blood passes through the placenta for the exchange of gases, nutrients,
558 electrolytes and waste products between the mother and fetus. Mutations in three genes,
559 [coagulation factor V \(F5\)](#), [fibrinogen, A alpha polypeptide \(FGA\)](#) and [thrombomodulin](#)
560 [\(THBD\)](#) (Quintero-Ronderos et al., 2017), were identified. These are involved in the
561 coagulation pathway. The flow of blood is necessary for the fetus to grow and any
562 disruption causing the blood to clot could result in loss of the pregnancy.

563

564 In summary, WES of POC or fetal DNA and parental DNA is a promising method to identify
565 variants in genes which might be responsible for RPL and/ or fetal abnormalities. Where
566 aberrations are inherited from the parents, a genetic diagnosis may provide invaluable
567 information for preimplantation screening or prenatal diagnosis in future pregnancies.
568 However, studies with larger unbiased cohorts are needed to conclusively determine
569 detection rates and the clinical utility of WES in this group of patients.

570

571 Chromosomal microarray analysis

572 In some cases, CNVs either as gains or losses may be responsible for pregnancy loss of a
573 fetus with an apparently normal karyotype. CNVs, both rare and common, may be impacting
574 pregnancy-related genes or pathways, resulting in pregnancy loss. These may involve single
575 genes or clusters of genes which are deleted, duplicated or disrupted.

576

577 Studies identified by our systematic review are summarised in Table III. Due to the diverse
578 approaches taken, the studies are difficult to compare collectively. Cohorts reported
579 sporadic pregnancy loss and RPL, different gestations and different methods of analysis.
580 Some studies (Bagheri et al., 2015, Rajcan-Separovic et al., 2010a, Rajcan-Separovic et al.,
581 2010b, Warren et al., 2009, Levy et al., 2014, Robberecht et al., 2012, Wang et al., 2017)
582 analysed both fetal tissue and parental DNA concurrently (i.e. a trio) to identify whether
583 CNVs were *de novo* or inherited. This is important in assessing both the likely pathogenicity
584 of the finding and the associated recurrence risk. Where the CNV is also detected in a
585 parent it is less likely to be causative of a pregnancy loss in isolation. It is possible that
586 inherited CNVs could still cause RPL where the CNV co-occurs with an autosomal recessive
587 gene mutation (SNV) on the other allele or where genes present within the CNV are relevant

588 to genomic imprinting or embryonic/ placental growth (Rajcan-Separovic et al., 2010a,
589 Rajcan-Separovic et al., 2010b).

590

591 Relatively little is known about the genes and pathways involved in pregnancy loss, and
592 therefore many CNVs identified will be classed as having uncertain clinical significance. One
593 study analysed CNVs in parents experiencing idiopathic RPL using functional enrichment
594 analysis, identifying biological pathways that were significantly over-represented, such as
595 antigen binding and immune signalling (Karim et al., 2017, Nagirnaja et al., 2014).
596 Enrichment was identified in genes associated with immunoregulatory interactions at the
597 feto-maternal interface and impaired immune signalling (Nagirnaja et al., 2014).

598

599 Identification of pregnancies with developmental abnormalities using hystero-embryoscopy
600 enables genetic abnormalities to be compared with developmental abnormalities and
601 growth disorganisation of the embryo. CNVs identified where there is a developmental
602 abnormality present are more likely to indicate genes important in early development. In
603 addition to evaluating a genetic cause for pregnancy loss, such studies can provide an
604 opportunity to identify and evaluate the function of the genes. Where variants are identified
605 in genes, through analysis of an enriched cohort, ~~such as this~~ [with developmental](#)
606 [abnormalities](#), it is easier to interpret their clinical significance.

607

608 Several studies explored the possibility of uniparental disomy (UPD) and looked for regions
609 of [Loss of heterozygosity](#) in euploid embryos (Levy et al., 2014, Robberecht et al., 2012,
610 Wang et al., 2017). The pathological relevance of UPD is difficult to evaluate as not all
611 platforms are capable of detecting UPD (eg. Oligo BAC array) and therefore are difficult to

612 compare. Pregnancy loss could be due to UPD resulting in unmasking of an underlying lethal
613 recessive disease gene(s) or imprinted genes.

614

615 CNVs were identified in the highly imprinted region 11p15.5. This region is abundant with
616 imprinted genes and has an important role in the maternal-fetal exchange. Aberrant
617 methylation or duplication of imprinted genes in this region could cause pregnancy loss
618 (Zhang et al., 2016).

619

620 Recurrent molar pregnancies

621 Although the majority of HM are sporadic, a small minority are recurrent and/or familial. A
622 number of studies looked at the role of genes including *NLRP7*, *C6orf221* (*KHDC3L*) and
623 *NLRP2* in pregnancy loss manifesting as recurrent molar pregnancy. In the cases reviewed,
624 the HM are euploid, and are instead caused by autosomal recessive mutations in genes
625 which code for the cell machinery that labels the parental origin of the two sets of
626 chromosomes.

627

628 It is thought that *NLRP7* and *C6orf221* are components of an oocyte complex that forms
629 during oogenesis and determines the epigenetic status of the oocyte genome by inactivating
630 genes. It is likely that mutations in *NLRP7* cause HM by impairing the normal imprinting
631 process causing maternal genes to be expressed when they should not be.

632

633 Studies have explored the role of *NLRP2*, [NLR family, pyrin domain-containing 5 \(*NLRP5*\)](#),
634 *NLRP7* and *C6orf221* in other forms of pregnancy loss such as partial moles, RPL, stillbirth,
635 infertility and multi-locus imprinting disturbance (Aghajanova et al., 2015, Andreasen et al.,

636 2013, Huang et al., 2013, Manokhina et al., 2013, Messaed et al., 2011, Docherty et al.,
637 2015). These have shown conflicting results, many showing no evidence of *NLRP7*, *NLRP2*
638 and *C6orf221* mutations in women with RPL (Aghajanova et al., 2015, Andreasen et al.,
639 2013, Manokhina et al., 2013).

640

641

642 Evidence from several papers suggests that genes involved in oocyte development,
643 maturation and epigenetic reprogramming are likely to be important in a subset of
644 pregnancy losses. One of the most studied epigenetic modifications is DNA methylation.
645 DNA methylation is implicated in the regulation of imprinting and the expression of
646 imprinted genes is thought to be important for the development and physiology of the
647 placenta (Frost and Moore, 2010). Aberrant DNA methylation of several imprinted loci ([H19](#),
648 [imprinted maternally expressed noncoding transcript \(H19\)](#), [long QT intronic transcript 1](#)
649 [\(LIT1\)](#) and [small nuclear ribonucleoprotein polypeptide N \(SNRPN\)](#)) was demonstrated in
650 pregnancy losses, with increasing methylation of these genes showing a positive correlation
651 with pregnancy loss. It is possible that inappropriate DNA methylation may either be a
652 contributing factor or consequence of the defect that led to pregnancy loss (Zheng et al.,
653 2013). It also remains to be investigated as to whether there are wider epigenetic defects at
654 other loci. Zheng et al. ([2013](#)) propose a multifactorial threshold model for pregnancy loss
655 where additional genetic and environmental factors may also play a role.

656

657 Other genetic causes

658 Mitochondria have been hypothesised to have an important role in development. They
659 predominantly regulate the production of ATP, used to regulate cellular metabolism.

660 Processes such as cell proliferation and development require high energy giving the
661 mitochondria an important role during pregnancy. Seyedhssani et al. (Seyedhssani et al.,
662 2010a, Seyedhssani et al., 2010b) have identified mutations in mtDNA in women with RPL
663 (Seyedhssani et al., 2010b). Furthermore a significant number of mutations were identified
664 in the D-loop of mtDNA. The D-loop contains essential elements for mtDNA transcription
665 and disruption could affect the transcription or translation of mtDNA, in turn compromising
666 embryonic development or causing pregnancy loss.

667

668 It is hypothesised that skewed XCI could be involved in the pathogenesis of RPL. Bagislar and
669 colleagues (Bagislar et al., 2006) demonstrated extremely skewed XCI in 17.7 % of patients
670 with RPL. It is suggested that skewed XCI could expose X-linked variants that are lethal in the
671 hemi-zygous state. In addition, a more recent review (Sui et al., 2015) included 12 case-
672 control studies on skewed XCI with or without RPL. In patients with RPL, skewed XCI was
673 significantly higher, although the significance drops with fewer losses and for less extreme
674 skewing. Although the association between RPL and skewed XCI is unclear, two mechanisms
675 have been proposed. Firstly, if a female carrier with a recessive lethal X-linked genetic
676 mutation and skewed XCI has a male fetus who inherits the X-linked genetic mutation, it
677 could lead to pregnancy loss. Secondly, an X-linked genetic mutation could cause follicular
678 atresia and an increase in aneuploid embryos resulting in pregnancy loss (Sui et al., 2015).

679

680 Six papers (Bendroth-Asmussen et al., 2016, McKie et al., 2014, Stouffs et al., 2011, Zhang et
681 al., 2016, Bhuiyan et al., 2008, Lopez-Carrasco et al., 2013) describe targeted sequence
682 analysis of specific candidate genes (*GBE1*, *RYR1*, *WNT6*, *DNMT3L*, *SYCP3*, *MSH4*, *HERG* and
683 *AURKB*) in either an individual case of pregnancy loss (Bendroth-Asmussen et al., 2016,

684 Bhuiyan et al., 2008) or in patient cohorts (McKie et al., 2014, Stouffs et al., 2011, Zhang et
685 al., 2016, Lopez-Carrasco et al., 2013). This targeting was informed by factors including
686 histopathological examination of placental tissue observed in fetal arrhythmia, scan findings
687 and functional prediction of gene pathways.

688

689 Limitations of current evidence

690 This review was completed in a systematic manner by two independent reviewers making it
691 reproducible. The limitation of this study, however, is the quality of the studies published to
692 date. Each study was scored according to our modified Newcastle-Ottawa scale
693 (Supplementary Table SIV) with a few of the studies being of poor quality and scoring as
694 little as 3 or 4 on our scale.

695

696 The most common limitations in these studies related to the small size of the studied
697 cohorts, with several focusing on a single family, and many of the studies lacking
698 information on control populations or statistical analysis. Work on small groups, and in
699 particular a single family, may detect genetic abnormalities that have occurred in isolation
700 or are very rare. In many cases this results in identification of variants in unique candidate
701 genes with no definitive causal effect. Therefore larger cohorts are needed to replicate
702 these findings and to determine how relevant these findings are to other couples with RPL.

703

704 There was also limited availability of functional data in many of the studies. A few studies
705 supplemented their cases with information on scan abnormalities or post-mortem
706 abnormalities detected in cases of losses and hystero-embryoscopy to correlate genetic

707 findings with findings in the embryo. The studies were also difficult to compare and collate
708 as there were multiple variations in the cohorts studied and the methods of analysis.

709

710 **Conclusion**

711 It is evident that there are many genetic and environmental factors that result in a
712 successful pregnancy and a disruption in any of these could contribute to pregnancy loss.

713 From the genetic perspective this includes both clearly pathogenic genetic causes, such as
714 sporadic aneuploidy and translocations, and other potential genetic causes such as smaller
715 CNVs and mutations in genes important in early fetal development. In addition, there are
716 likely to be complex genetic contributions, such as multi-factorial inheritance, and changes
717 in methylation (epigenetics) and mitochondrial function, which could be contributing to
718 pregnancy loss. These more complex genetic mechanisms may be influenced by
719 environmental factors, such as diet, medication, pollutants and lifestyle, which could
720 provide a cumulative effect resulting in pregnancy loss.

721

722 The papers we have identified have demonstrated that monogenic aetiologies could
723 contribute to a proportion of pregnancy losses. However, as most studies have been carried
724 out in highly selected families or small cohorts, additional studies are required to further
725 assess if this technology is generalisable to more couples experiencing RPL.

726

727 It is plausible that cases of pregnancy loss (particularly in RPL) may have causative mutations
728 not detectable with routine cytogenetic analysis or fetal scans, but are detectable by WES.
729 Although WES is not currently recommended for routine diagnostic use for pregnancy
730 losses, the identification of genes associated with pregnancy loss will be of significant

731 individual patient impact with respect to treatment and availability of PGD. If monogenetic
732 etiologies of RPL and the overall prevalence of monogenetic causes of pregnancy loss are
733 better elucidated through larger, well-designed studies, the identification of non-aneuploid
734 causes of RPL could be of significant patient impact.

735

736 Knowledge of specific genes that contribute to pregnancy loss could also be of importance
737 in understanding the biological pathways that can cause pregnancy loss. However, much
738 larger and more comparable cohort studies are required in all of these areas to determine
739 causality of candidate genes and to dissect out these effects, as at present many of these
740 findings are of uncertain clinical significance. Functional analysis, such as embryoscopy
741 studies and *in vivo* animal modelling, may assist in further assessment of the mutation effect
742 on early embryonic development.

743

744 RPL is a complex problem influenced by many different aetiologies. Currently, with the
745 exception of aneuploidy and other chromosomal abnormalities, routine investigation for the
746 genetic contributions causing pregnancy loss is limited. With increased knowledge of
747 additional non-aneuploid contributions to RPL, additional genetic testing recommendations
748 may be made in the future to couples experiencing RPL. These would have implications for
749 diagnosis and recurrence risks.

750

751 **Authors' roles**

752 EC- Study search, study selection, data extraction, quality assessment and writing.

753 SH- Data extraction, quality assessment and editing

754 PS- Study design, critical appraisal of manuscript

755 NM- Critical appraisal of manuscript and editing

756 AC- Study design and critical appraisal of manuscript

757 SA- Supervision, study selection, writing and editing

758

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761

762 Conflict of interest

763 There are no conflicts of interest to declare.

764

765

766 References

767 **AUTHOR: please would you recheck journal style for the references and edit accordingly?**

768 **Thank you (e.g. upper/lower case, bold text).**

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1042 **Figure legend**

1043 **Figure 1** PRISM flow diagram for a systematic review of the potential genetic causes of
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