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Diagnostic yield of exome sequencing in fetal growth restriction: Systematic review and meta-analysis

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SHORT TITLE: *Exome sequencing in fetuses with fetal growth restriction*

KEYWORDS: diagnostic yield; exome sequencing; NGS, fetal growth restriction; prenatal diagnosis, meta-analysis

Contribution

What are the novel findings of this work?

Our study provides the first systematic review and meta-analysis of the existing evidence on the diagnostic yield of exome sequencing in fetuses with isolated fetal growth restriction. It revealed that next generation sequencing analysis provides a 12% incremental yield in fetuses with isolated fetal growth restriction and normal chromosome analysis.

What are the clinical implications of this work?

According to our results when an amniocentesis is performed in fetuses with growth restriction in absence of structural fetal anomalies and chromosomal microarray analysis is normal, exome sequencing should be considered.

ABSTRACT

Objective To determine the diagnostic yield of exome sequencing (ES) above that of chromosomal microarray analysis (CMA) or karyotyping in fetuses with isolated fetal growth restriction (FGR)

Method This was a systematic review conducted in accordance with PRISMA guidelines. Selected studies included those with: (a) only fetuses with FGR in absence of fetal structural anomalies and (b) negative CMA or karyotyping result. Only positive variants classified as likely pathogenic or pathogenic determined to be causative of the fetal phenotype were considered. A negative CMA or karyotype result was treated as the reference standard. Incidence was used as the pooled effect size by single-proportion analysis using generalized linear mixed model (by logit transformation).

Results Eight studies with data on ES diagnostic yield, including 146 fetuses with isolated FGR, were identified. Overall, a pathogenic variant determined to be potentially causative of the fetal phenotype was found in 17 cases, resulting in a 12% (95% CI: 7-18%) incremental performance pool of ES.

Conclusion. A monogenic disorder was prenatally found in association ~~with 10% of fetuses~~ with apparently isolated FGR in 12% of those fetuses.

INTRODUCTION

Fetal growth restriction (FGR) is simply defined as an estimated fetal weight (EFW) below the 10th percentile for gestational age by the Society for Maternal-Fetal Medicine (SMFM) (1). Discordantly, the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) (2) defines FGR as severe or Doppler-abnormal fetal smallness, ~~differently to the condition named "small-for gestational age"~~ including only fetuses with an EFW below the 3rd percentile alone, or those with an EFW between the 3rd and 10th percentile associated with abnormal Doppler studies. FGR has been described in association with several adverse perinatal outcomes together with an increased risk of neurovelopmental delay (3)(4). FGR is commonly caused by placental insufficiency (5)(6), although structural and genetic abnormalities (7) and congenital infections, has also been considered in the etiology of FGR. Several national guidelines, including the SMFM and the Royal College of Obstetricians and Gynecologists (8) recommend prenatal genetic diagnostic testing in cases of early onset FGR or in FGR associated with structural anomalies, because an increased likelihood of a genetic disorder in these cases. ~~However, a Delphi consensus of experts in 2016, and subsequently~~ Another discrepancy in the definition of FGR is the presence of congenital defects, which are considered an exclusion criteria only by ISUOG (9)(10)~~, maybe because a genetic disorder in a growth restricted fetus remarkably worsen its prognosis~~.

Chromosomal microarray analysis (CMA) has been increasingly offered in early severe FGR, showing a 5-10% incremental diagnostic above the karyotype (11) (12), but advanced genetic testing is scarce and may result in an underestimation of the real prevalence of monogenic diseases in this population (4). Exome-sequencing (ES) enables the assessment of the coding regions of more than 20,000 genes of the human genome. Although it covers approximately 1-2% of the genome, ES is able to assess 85% of known disease-causing variants. Prenatally, ES has been shown to be a powerful diagnostic test in fetuses with structural anomalies (13) (14) (15) The establishment of a timely molecular diagnosis makes it possible to offer genetic counseling and has significant value for prenatal and perinatal medical management, as well as allowing couples to make future family-planning decisions.

In this study, a systematic review of the literature and meta-analysis were performed to assess the ES diagnostic yield in fetuses with an apparently isolated FGR and a negative result at CMA or karyotyping. This study would add to the literature and help with counseling for patients in this scenario, since only a couple of sub-analysis address this issue so far (16)(17).

METHODS

Protocol and registration

The study protocol was registered prospectively at the OSF (Open Science Framework) which is a prospective database for protocols and scientific projects under the following DOI: 10.17605/OSF.IO/MEZC3) and PROSPERO number CRD42022364710. There is no need for institutional approval in our center in the case of systematic reviews and meta-analyses. This meta-analysis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for randomized controlled trials (18).

Eligibility criteria

Included in this systematic review were observational studies of pregnancies with the following: (a) singleton pregnancies; (b) only fetuses with isolated FGR, defined as absence of fetal structural defects except hypospadias (given that it appears to be associated with placental insufficiency) (19), although soft fetal markers or ultrasound signs were accepted, and (c) negative CMA or karyotyping result. Only positive variants classified as likely pathogenic or pathogenic determined to be potentially causative of the fetal phenotype were considered. ES can be applied using a solo (the fetus alone was sequenced) or a trio (both parents and fetus were sequenced) approach, and both were included in this review. The following studies were excluded: case reports, opinion articles or letters, series in which gene panels were applied, series with cases with an identified disease in the family history; and series from which data could not be extracted and the corresponding author did not provide additional information.

Information sources and search

A systematic search was conducted using PubMed database and Ovid Medline to identify relevant published manuscripts without time limit on the date of publication. References of relevant publications were searched manually for any additional potentially relevant published studies. We performed three systematic searches, two were based on ES and FGR and the last one was an exome search on fetal diagnosis. The searches ware run on 22 December 2022. Details of the searches and the MeSH terms used are given in Figure 1. This meta-analysis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for randomized controlled trials.

Study selection

Abstracts identified as relevant were assessed by two independent evaluators (M.P. and A.B.). If the studies complied with the inclusion criteria, full-text articles were reviewed.

Data collection process and data items

The following data were extracted onto a datasheet: study period, site and country where the study was carried out, study inclusion criteria, sample size, number of fetuses with isolated FGR, presence of ultrasound signs or soft markers and Doppler studies, interpretation approach for ES (whole/clinical ES, solo/trio approach) or whole genome sequencing, Sanger sequencing validation, criteria for variant classification, and ES results and positive diagnoses.

Outcome measures

The primary outcome was the NGS diagnostic yield in fetuses with apparently isolated FGR and normal chromosomes as assessed by karyotyping, QF-PCR and/ or CMA.

Assessment of risk of bias

The quality of the included studies was assessed according to the Standards for Reporting of Diagnostic Accuracy (STARD) criteria, modified for this project. The quality criteria deemed most important to optimize accuracy were the following: (a) fetal phenotype described in detail; (b) study including only "isolated FGR" (FGR in absence of fetal structural defects); (c) a prospective series; (d) ES trio analysis (both parents and fetus); (e) homogenous previous genetic testing (i.e. CMA/karyotyping) in the whole series; (f) Sanger sequencing validation; (g) variants classified according to the American College of Medical Genetics and Genomics (ACMG) criteria (20); (h) Incidental findings reported; and (i) variants of uncertain significance reported. The risk of bias was measured individually by two reviewers (M.P. and A.B.).

Strategy for data synthesis and statistical analysis

The extracted results were pooled in a meta-analysis. For the primary outcome, the diagnostic yield was used as the pooled effect size by single-proportion analysis using ~~random-effects modeling~~ generalized linear mixed model (by logit transformation) (21) (22) a continuity correction analysis was performed for cells with zero values. Between-study heterogeneity/variability was assessed using the tau^2, ~~χ^2 (Cochrane Q)~~ and I^2 statistics. Publication bias was assessed using Egger's test. Results were assessed using forest plot and

presented as proportions. Statistical analyses were conducted using R studio v1.0.136 (The R Foundation for Statistical Computing; meta v4.2 package (23).

RESULTS

Initially 1085 studies were selected initially from PubMed and Ovid Medline, using either ES or whole genome sequencing to prenatally study fetuses with isolated FGR, considered as such those FGR cases with no apparent fetal structural anomalies. Among the 110 abstracts selected at that search, 25 articles were reviewed fully, of which eight were deemed eligible for inclusion in the study (13) (14) (24) (25) (26) (27) (28) (29) (Figure 1). Gabriel et al. provided us with detailed data for this meta-analysis. Two series previously reported (13) (14) were recently updated by their authors and published in a meta-analysis by Mone *et al*. (16). The number of isolated FGR cases selected per study ranged from one to 51 fetuses. Only one of these series included FGR cases exclusively. Four studies were conducted in China, two in the United Kingdom, one in the United Stated and one in Germany (Table 1).

Among the eight selected studies for this systematic review, whole ES was performed in four and in the remaining four series the morbid OMIM genes alone were studied (clinical exome interpretation approach). In seven series proband and parental samples were simultaneously analyzed (trio), and in one the proband samples were studied alone (solo-ES) (25). Deep description of phenotype of individual fetuses was lacking in two studies (14)(13)(Figure 2). Data on additional ultrasound findings and Doppler studies was provided in two series (28) (29), while gestational age at diagnosis was indicated in three series (24) (27) (28). Among the 156 selected fetuses with FGR and no structural anomalies, there were 143 (92%) fetuses with no further ultrasound findings, while in the remaining 13 some ultrasound signs were observed (Table 2), being olygohydramnios (n=7) the sign most commonly found.

The yield of pathogenic variants among fetuses with isolated FGR and normal chromosomes, according to the random-effects model, was 12% (95% CI, 7-18%; I^2=0%, Tau^2; 0) (Figure 3). There was no significant heterogeneity among studies as showed by Tau^2 and I^2. Egger's test for publication bias showed no significant results (bias: -1.003; p=0.735). Interestingly, enough, additional ultrasound findings, including oligohydramnios, were not reported in none of the 17 cases with a pathogenic/likely pathogenic variant.

Among the 17 FGR cases in which ES found the causative variant, there were 8 cases with a dominant inheritance pattern, 4 autosomal recessive, two x-linked and in the remaining two the variant and gene were not described (Table 3). Among the cases with a dominant inheritance pattern, there were three Cornelia-deLange syndrome cases, from the same series, one achondroplasia, one osteogenesis imperfecta or Ehnlers-Danlos syndrome, all diagnoses

performed in absence of further ultrasound findings. Among the recessive cases, one had two compound heterozygous variants in the *IARS1*gene (27) This gene is associated with growth restriction, impaired intellectual development, hypotonia, and hepatopathy (GRIDHH)~~(# 617093 OMIM). Both variants were missense variants NM_013417.2, c.2975A>G, (p.Asn992Ser), and NM_013417.2: c.2420C>G (p.Pro807Arg) not previously described, neither in the ClinVar database, nor in the general healthy population. According to the ACMG criteria they were classified as likely pathogenic. Segregation studies confirmed that one variant was inherited from the mother and the other from the father. Since GRIDHH has a recessive inheritance, and the variants were in *trans*, the second variant was classified as a likely pathogenic variant.~~ The single causative variant found in homozigosity was the one found in the *SKIV2L* gene, associated to achondroplasia, parentally inherited.

Two incidental variants were reported only in one series (29), and 11 variants of uncertain significance in two series (26) (29). In addition, there were two uniparental disomies included in the series of Gabriel et al. that were excluded in our review since they were not found at ES.

DISCUSSION

This systematic review and meta-analysis of fetuses with apparently isolated FGR, meaning FGR in absence of fetal structural anomalies except hypospadias, and normal chromosomes at CMA or karyotyping demonstrates a 12% (95% CI, 7-18%) ES diagnostic yield. It is of paramount importance to highlight that prenatally any isolated finding should be considered as "apparently isolated" until confirmation after birth. Given that in late FGR the indication of ES is established when additional phenotype findings are observed, this may lead to prenatally miss some genetic syndromes. This 12% yield is higher than that found in a previous meta-analysis by our group in CMA applied to isolated FGR and normal karyotype (30), since a 4% yield was observed in isolated FGR.

This is the first proper meta-analysis on NGS in FGR, since only a couple of stratified sub-analysis within a meta-analysis of fetal structural anomalies have been reported showing a coincidental 4% yield: one positive among 28 isolated FGR cases (17), and 7 positives among 70 isolated FGR cases (16). The huge discrepancy observed between our results and those obtained by the last sub-analysis can be explained by the use of different statistical methodology that lowers a 10% arithmetical yield to a 4% pooled yield (15). Noteworthy, the two more recently reported series have the largest sample size (with 43 and 51 isolated FGR cases) and showed the highest diagnostic yield (14% and 16%) (29)(28).

Interestingly enough, only four of the positive cases were affected by one of the 17 most common syndromes found in association with FGR (three Cornelia deLange syndromes from the same series and one achondroplasia) according to a recent review by our group (4). Among the cases with a dominant inheritance, in addition to the three Cornelia-deLange syndromes and one achondroplasia, there were two cases with an alteration in the collagen genes: *COL1A1* gene (osteogenesis imperfecta or Ehlers-Danlos syndrome) and *COL2A1* (syndrome not described). It has been reported that 11% of children with familial short stature present an abnormality in the collagen genes (31). It has to be kept in mind that since this meta-analysis only includes apparently isolated FGR, all the genetic diagnoses were established in absence of additional ultrasound findings. In addition, there is one gene, the *EP300*, not typically found in FGR, that may be associated with more than one condition, which was not identified in the corresponding series. Another gene, the *TBX* gene, related with diGeorge syndrome, was reported to be associated with short stature in adulthood in 20% of the cases. Among the cases with autosomal recessive inheritance patterns, variants were found in the *SLC25A13* and

GNPTAB genes, related to metabolic disorders with impaired growth in affected children. Finally, there were 2 X-linked genes, presenting one a dominant inheritance and the other a recessive inheritance.

Whether a diagnosis of a genetic disorder in growth restricted fetuses should be considered an exclusion criterion in the definition of FGR, as stated by ISUOG guidelines, appears to us controversial, because it is based on the assumption that the "intrinsic" growth potential in genetic syndromes is decreased, while on contrary, in real FGR there is an extrinsic cause preventing those fetuses to achieve their intrinsic growth potential. This assumption is challenged by the finding of abnormal third trimester Doppler studies in fetuses with common trisomies that account for 90% of fetuses with a trisomy 13, 55% in trisomy 18 and 75% in trisomy 21(32)(33), signaling that placental insufficiency may also be present in pregnancies affected by a genetic disorder.

The contribution of chromosomal anomalies as a cause of FGR was established in 1993 to account for 19% by Snijders et al. (34), being trisomy 18 and triploidy the anomalies most commonly found. More recently, in apparently isolated FGR this yield was found to decrease to 6.4%, probably because pregnancies have been already screened for aneuploidy in the first trimester (35). Regarding pathogenic microdeletions and microduplications, a systematic review and meta-analysis by our group showed in 2018 that the incremental yield over karyotyping in non-malformed growth-restricted fetuses observed with the use of CMA was 4% (95%CI: 1-6%) (30), being 22q11.2 duplication, Xp22.3 deletion, and 7q11.23 deletion (Williams-Beuren syndrome) the most frequently found pathogenic CNVs.

The SMFM in 2020 recommended pregnant women be offered prenatal diagnostic testing with CMA when unexplained isolated FGR is diagnosed below 32 weeks of gestation (1). However, there are no recommendations on ES testing in fetuses with late FGR. Initially, guidelines of scientific societies recommended the use of ES in the prenatal setting only in selected fetal anomalies highly suggestive of a genetic disorder when more specific test driven by the fetal phenotype failed to determine a diagnosis. More recently, the ACMG stated that ES may be considered for a fetus with any ultrasound anomaly when standard CMA and karyotype analysis have failed to yield a definitive diagnosis, with the exception of cases in which a specific diagnosis is suspected, in which case, molecular testing for the suggested disorder (with single-gene test or gene panel) should be the initial test (36). Similarly, a joint-position statement (2018), from the International Society for Prenatal Diagnosis, the Society for

Maternal and Fetal Medicine and the Perinatal Quality Foundation, suggested that fetal sequencing may be beneficial in cases with a single major anomaly or with multiple-organ-system anomalies that are suggestive of a possible genetic etiology, but FGR is again not mentioned (37).

There were also some limitations of the review processes used. The first limitation was due to the fact only one of the selected studies include FGR cases alone, which made data extraction cumbersome. The second limitation was related to the fact that FGR and isolated FGR are not defined in some studies, and when defined they are not concordant. Therefore, we strictly looked at the phenotype description, and only fetuses with no additional structural defects (except hypospadias) were considered, while ultrasound signs and soft markers could be present. The third limitation is the lack of information on the gestational age and Doppler studies in most of the series and in most of the positive cases, data that would be relevant to confirm whether fetuses with early FGR and normal Doppler have an increased likelihood of a genetic origin. In positive cases, at least, gestational age was available for 15 of the 17 cases, revealing that all but one, ES was performed before 32 weeks (early-onset FGR).

This systematic review and meta-analysis have shown that ES applied to fetuses with apparently isolated FGR enables identification of the causative gene in 12% of cases.

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Figure 1 Flowchart summarizing inclusion in the systematic review of studies reporting on ES diagnostic yield in fetuses with isolated FGR with a negative chromosome analysis (CMA or karyotype) and no previous family history.

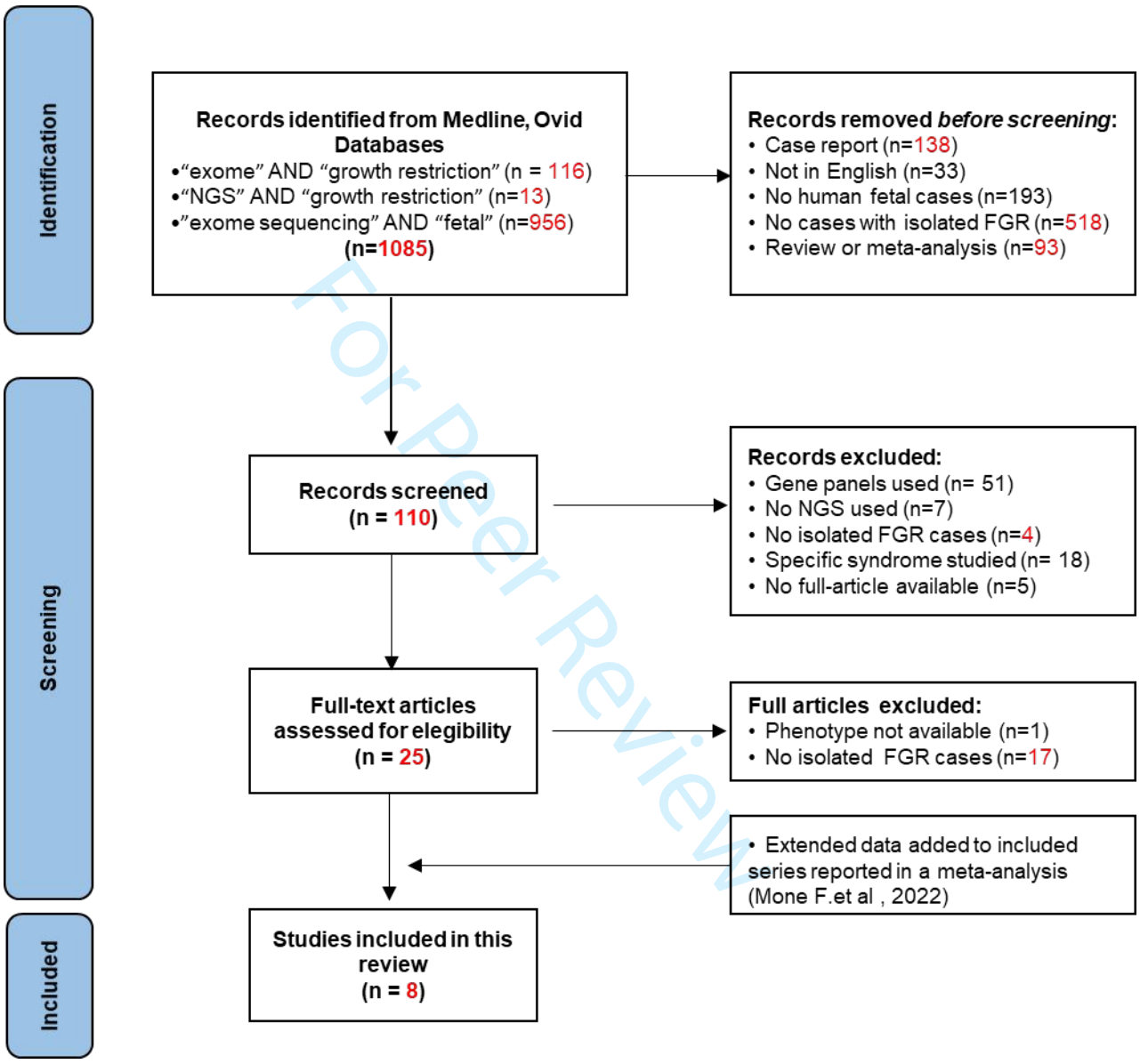


Figure 2 Quality assessment of the eight studies included in this systematic review

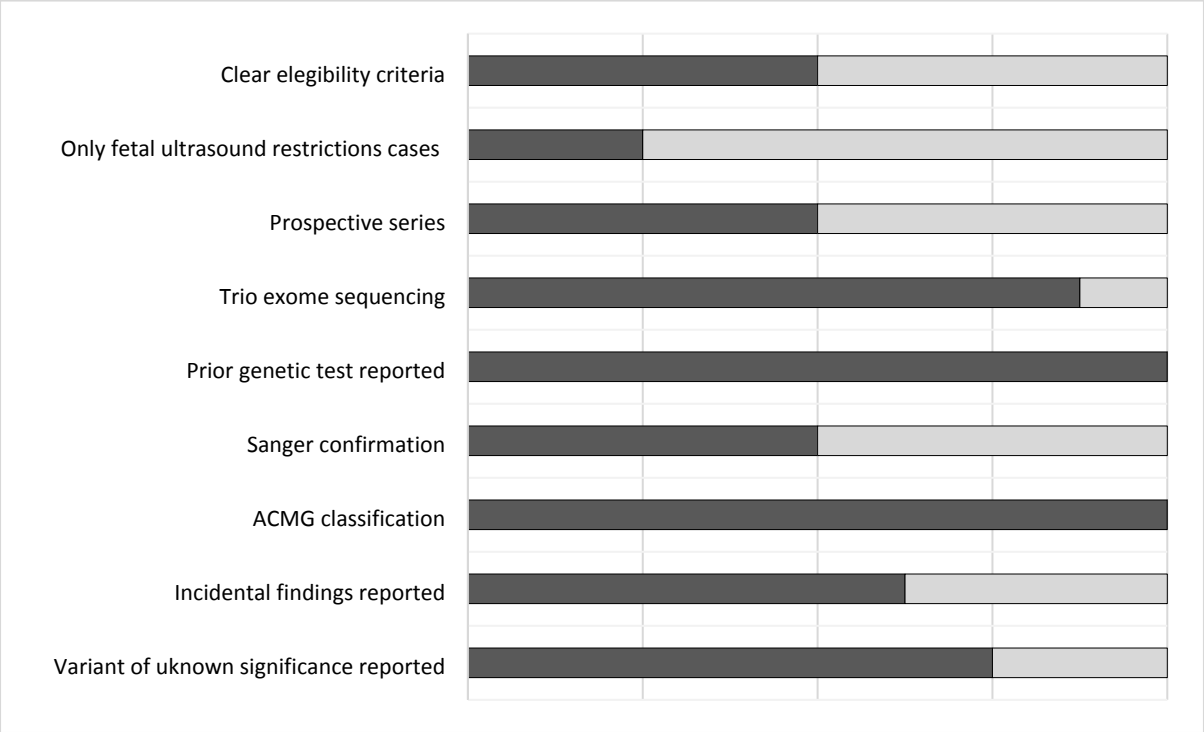


Figure 3: Forest plot demonstrating pooled incremental yield of prenatal ES in isolated FGR

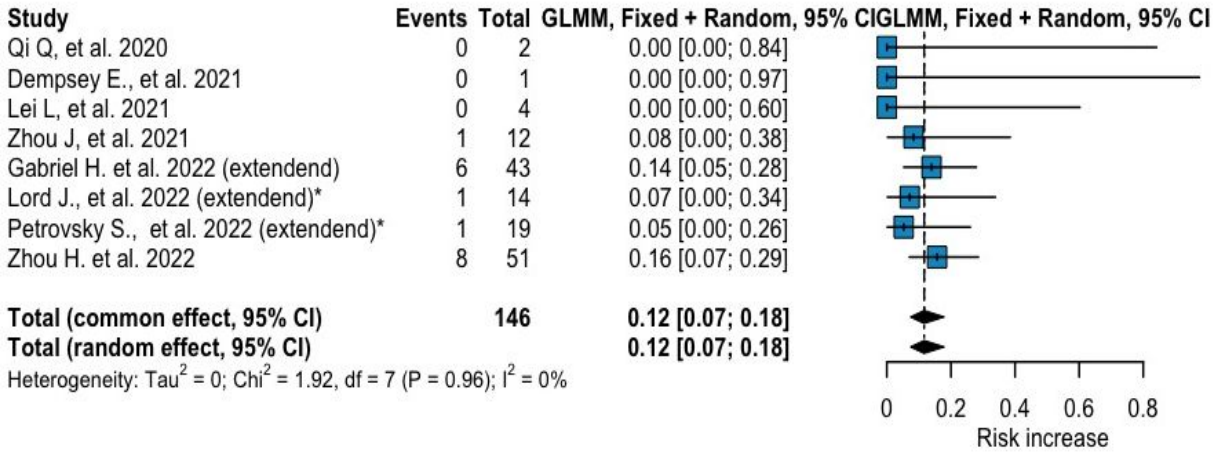


Table 1 Characteristics of the eight studies included in this systematic review and meta-analysis

Author	Year	Site	Fetuses with Isolated FGR	Definition of FGR	Interpretation approach	Positive diagnoses
Qi Q *et al. (24)*	2020	Beijing, China	2	Early-onset fetal growth restriction	Trio-CES	0
Dempsey E *et al. (25)*	2021	London, UK	1	Severe symmetrical IUGR.	Solo-CES	0
Lei L *et al. (26)*	2021	Chongqing, China	4	Not defined	Trio-CES	0
Zhou J *et al. (27)*	2021	Shanghai, China	12	Not defined	Trio-CES	1
Gabriel H *et al. (extendend)(28)*	2022	Tübingen, Germany	43	EFW<10th percentile	Trio-WES	6
Lord J *et al. (extendend)*(13)*	2022	Hinxton, UK	14	EFW<10th percentile + placental insufficiency signs*	Trio-WES	1
Petrovsky S *et al. (extendend)*(14)*	2022	New York, NY, USA	19	EFW<10th percentile + placental insufficiency signs*	Trio-WES	1
Zhou H *et al. (29)*	2022	Guangzhou, China.	51	EFW<3rd percentile	Trio-WES	8

Only first author of each study is given. CES: clinical-exome sequencing (exome sequencing restricted to analysis of coding sequences of the OMIM morbid genes) WES: whole exome sequencing; EFW: estimated fetal weight; * data extracted from Mone F. et al. (2022) meta-analysis (16)

Table 2. How fetuses with apparently isolated fetal growth restriction are described in each of the studies included in this review

Phenotype		N
Qi Q. *et al. (24)*	Early-onset FGR	2
Dempsey E. *et al. (25)*	Severe symmetrical IUGR	1
Lei L. *et al. (26)*	IUGR	4
Zhou J. *et al.(27)*	Isolated FGR or severe isolated FGR	9
	FGR and abnormal middle cerebral artery peak systolic velocity	1
	FGR and suspected hypospadias	1
	FGR, oligoamnios and abnormal middle cerebral artery peak systolic velocity	1
Gabriel H. *et al. (extended)(28)*	IUGR, reverse flow of A. umbilicalis, brain sparing ACM	1
	IUGR and single umbilical artery	2
	IUGR and oligohydramnios	7
	IUGR	33
Lord J. *et al. (extendend)* (13)*	Isolated FGR	14
Petrovsky S. *et al. (extendend)* (14)*	IUGR	19
Zhou H. *et al. (29)*	FGR with an estimated fetal weight below the third percentile	51

FGR: fetal growth restriction; IUGR: Intra-uterine growth restriction; * data extracted from the meta-analysis of Mone F. *et al.*(2022).

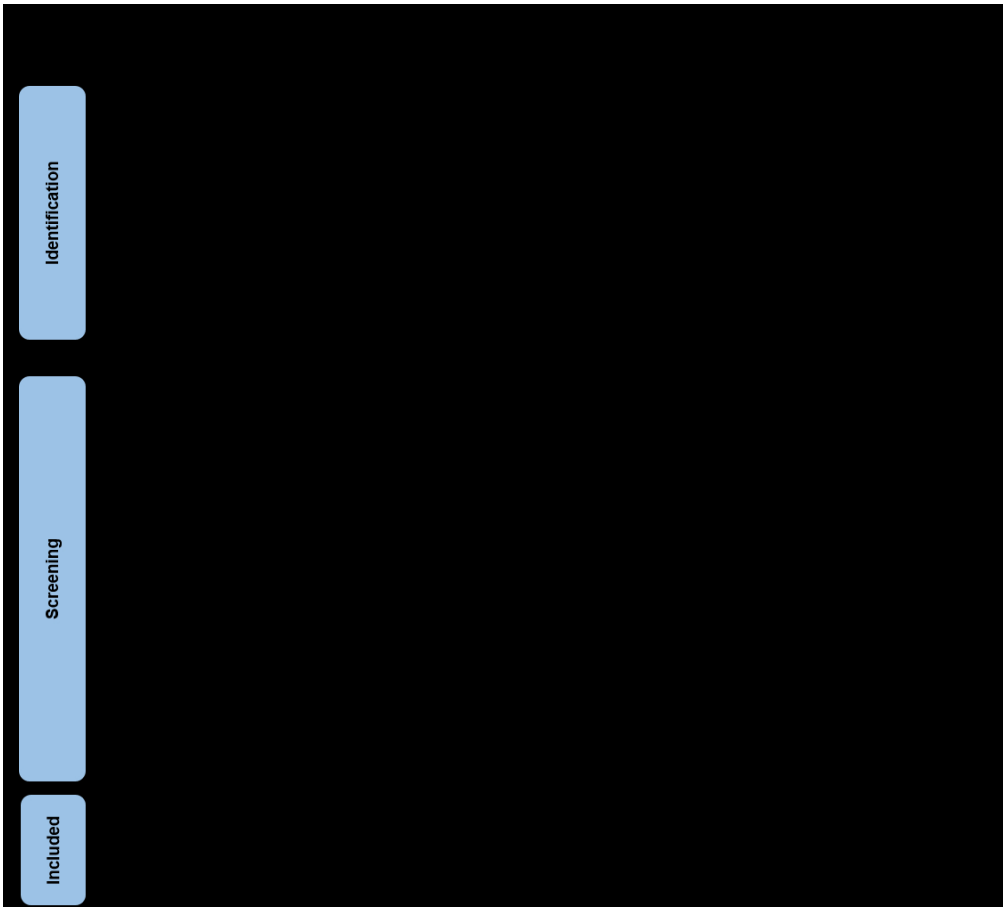
Table 3. Pathogenic or likely pathogenic variants and associated monogenic diseases identified in fetuses with a diagnosis. Data of one of the Petrovsky's cases and one of Lord's cases were not reported

Author	Phenotyphe. (Gestational Age at diagnosis)	Variant	Gene	Zygosity	Origin	Associated condition	Inheritance	# OMIM
Zhou J. *et al*	Isolated FGR (22+6 wk.)	NM_013417.2 c.2420C>G, p.Pro807Arg (Mat); NM_013417.2, c.2975A>G, p.Asn992Ser (Pat)	*IARS*	Compound heterozygous	Novel	Growth retardation, impaired intellectual development, hypotonia, and hepatopathy (GRIDHH)	AR	617093
Gabriel H. et al.	Isolated FGR (28+4 wk.)	c.[2T>C];[1399C>T], p.[?];[Arg467*]	*SLC25A13*	Compound heterozygous	Not specified	Citrullinemia, type II, neonatal-onset	AR	605814
	Isolated FGR (24+1 wk.)	Not specified	*GNPTAB*	Not specified	Not specified	Mucolipidosis II alpha/beta or Mucolipidosis III alpha/beta	AR	-
	Isolated FGR (21+2 wk.)	Not specified	*COL2A1*	Heterozygous	Not specified	Not specified	AD	-

	Isolated FGR (25 wk.)	Not specified	*EP300*	Heterozygous	Not specified	Not specified	AD	-
	Isolated FGR (22 wk.)	Not specified	*TBX1*	Heterozygous	Not specified	DiGeorge syndrome or Velocardiofacial syndrome	AD	-
	Isolated FGR (34+4 wk.)	Not specified	Not specified	Not specified	Not specified	-	-	-
Zhou H.*et al*	Isolated FGR (<32 wk.)	NM_133433.3. c.7789delC (p. Leu2597CysfsTer14)	*NIPBL*	Heterozygous	*de novo*	Cornelia de Lange syndrome 1	AD	122470
	Isolated FGR (<32 wk.)	NM_133433.3 c.7012G>C (p. Ala2338Pro)	*NIPBL*	Heterozygous	*de novo*	Cornelia de Lange syndrome 1	AD	122470
	Isolated FGR (<32 wk.)	NM_133433.3 c.6983C>A (p. Thr2328Lys)	*NIPBL*	Heterozygous	*de novo*	Cornelia de Lange syndrome 1	AD	122470
	Isolated FGR (<32 wk.)	NM_000284.3 c.1142_1145dupATCA (p. Trp383SerfsTer6)	*PDHA1*	Heterozygous	*de novo*	Pyruvate dehydrogenase E1-α deficiency	XLD	312170
	Isolated FGR (<32 wk.)	NM_00112789 8.3 c.934-1G>T	*CLCN5*	Hemizygous	Maternally inherited	Dent disease type I	XLR	300009

	Isolated FGR (<32 wk.)	NM_006929.4 c.1120C>T (p. Arg374Ter)	*SKIV2L*	Homozygous	Parentally inherited	Tricho-hepato-enteric syndrome	AR	614602
	Isolated FGR (<32 wk.)	NM_001163213.1 c.1144G＞A (p.Gly382Arg)	*FGFR3*	Heterozygous	*de novo*	Achondroplasia	AD	100800
	Isolated FGR (<32 wk.)	NM_000088.3 c.2362G>A (p. Gly788Ser)	*COL1A1*	Heterozygous	Paternally inherited	Osteogenesis imperfecta or EhlersDanlos syndrome	AD	-

AR: autosomal recessive inheritance; AD: autosomal dominant inheritance; XLD: X-linked dominant inheritance; XLR: X-Linked recessive inheritance



Flowchart summarizing inclusion in the systematic review of studies reporting on ES diagnostic yield in fetuses with isolated FGR with a negative chromosome analysis (CMA or karyotype) and no previous family history.

190x171mm (150 x 150 DPI)

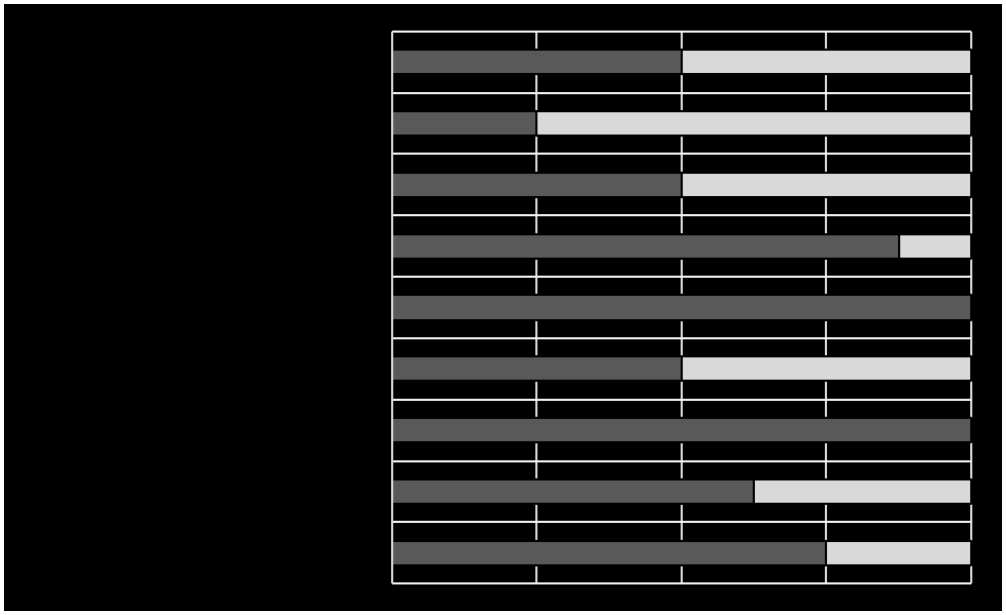


Figure 2 Quality assessment of the eight studies included in this systematic review

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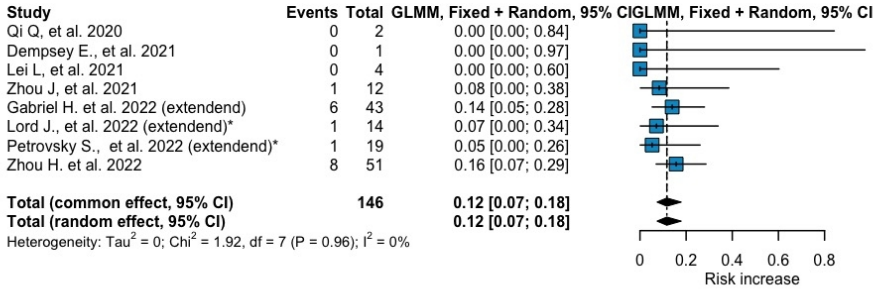

Figure 3: Forest plot demonstrating pooled incremental yield of prenatal ES in isolated FGR

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