Should we offer prenatal exome sequencing for intrauterine growth restriction or short long bones? A systematic review and meta-analysis

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OBJECTIVE: This study aimed to determine the incremental yield of prenatal exome sequencing over chromosomal microarray or G-banding karyotype in fetuses with: (1) intrauterine growth restriction related to placental insufficiency or (2) short long bones, in isolated and nonisolated instances for both scenarios.

DATA SOURCES: Data were collected via electronic searches for relevant citations from January 2010 to April 10, 2022 in MEDLINE, Embase, Web of Science, and Cochrane, and using relevant bibliographies and data generated in-house.

STUDY ELIGIBILITY CRITERIA: Included were prospective or retrospective cohort studies and/or case series with: (1) n>5 cases of short long bones and/or intrauterine growth restriction undergoing prenatal sequencing with a clearly defined phenotype including assessment of placental function; (2) testing based on prenatal phenotype only; (3) a nondiagnostic chromosomal microarray/karyotype; and (4) known results of genetic testing.

METHODS: Incremental yield was calculated for each study and as a pooled value for the aforementioned groups using a random-effects model. Results were displayed in forest plots with 95% confidence intervals. Heterogeneity was assessed statistically using Higgins' I^2. Publication bias was assessed graphically using funnel plots. Quality assessment was performed using modified Standards for Reporting of Diagnostic Accuracy criteria (International Prospective Register of Systematic Reviews registration number CRD42022324680).

RESULTS: Nineteen studies were included (n=452 cases). The apparent incremental yields with prenatal sequencing were: (1) 4% (95% confidence interval, −5.0 to 12; I^2=0%) in isolated intrauterine growth restriction with evidence of placental insufficiency, (2) 30% (95% confidence interval, 13–47; I^2=1%) in intrauterine growth restriction with additional structural anomalies, (3) 48% (95% confidence interval, 26–70; I^2=73%) in isolated short long bones, and (4) 68% (95% confidence interval, 58–77; I^2=51%) in short long bones with additional skeletal anomalies. Of the 37 short long bone cases with a diagnosis, 32 had a skeletal dysplasia, with thanatophoric dysplasia and osteogenesis imperfecta being the most common (both 21.6% [n=8/37]). In fetuses with short long bones and additional skeletal features, osteogenesis imperfecta was the most common diagnosis (28% [n=57/204]). Where documented, the inheritance patterns were de novo in 75.4% (n=150) of cases.

CONCLUSION: Prenatal sequencing adds substantially to incremental yield over chromosomal microarray in fetuses with short long bones or multisystem intrauterine growth restriction. Robust studies are required to assess the utility of fetal sequencing in isolated intrauterine growth restriction with evidence of placental insufficiency, which cannot be recommended on the basis of current evidence.

Key words: anomaly, exome sequencing, intrauterine growth restriction, next-generation sequencing, prenatal, short long bones, skeletal dysplasia, small-for-gestational-age

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AJOG at a Glance

Why was this study conducted?

This study was conducted to facilitate development of inclusion and exclusion criteria for prenatal exome sequencing.

Key findings

Prenatal exome sequencing has limited incremental yield over standard karyotyping and microarray analysis in fetuses with isolated intrauterine growth restriction, with moderate yields in intrauterine growth restriction with multisystem abnormalities and isolated short long bones, and a high yield for short long bones associated with other skeletal anomalies.

What does this add to what is known?

Short long bones may be considered as an indication for prenatal exome sequencing, but the incremental yield in isolated intrauterine growth restriction with evidence of placental insufficiency is very low and more evidence is required.

Introduction

Exome sequencing is a novel genomic technology that has the ability to interrogate the human genome to the resolution of a single nucleotide, enabling screening of multiple genes in 1 test. In prenatal diagnosis, the yield (up to 80%) for identifying a unifying genetic cause when applied in the presence of structural fetal anomaly supersedes that of standard chromosome microarray (CMA), which is limited to identifying copy number variation in the form of microdeletions and microduplications.[1] Following publication of the 2 largest prospective series in which prenatal exome sequencing was conducted for unselected fetal anomalies,[2,3] its provision has been translated into clinical practice to enable discovery of a unifying prenatal genetic diagnosis in some high-income countries.[4,5] Remaining an expensive and limited resource, such translation and supporting research have uncovered approaches that can optimize diagnostic yield. These include: (1) case selection by a clinical geneticist as part of a multidisciplinary team review; (2) detailed prenatal phenotyping; (3) CMA in parallel; and (4) evidence-based inclusion and exclusion criteria such as those used in the National Health Service (NHS) England rapid prenatal sequencing pathway.[6]

Although the yield of prenatal sequencing in fetal structural anomalies affecting multiple systems and skeletal dysplasias has been well documented,[2,3,7–11] its use in cases of isolated intrauterine growth restriction (IUGR) associated with placental insufficiency or in cases of isolated short long bones has not been well described in the literature.[11] This is compounded by the fact that IUGR secondary to placental insufficiency frequently presents with short long bones, making assessment of placental function a critical part of the phenotypic workup.[12] For diagnosis of placental insufficiency, we remain reliant on screening tools such as uterine artery and fetal Doppler studies and maternal biomarkers, which are by nature nondiagnostic.[13] The limitations of prenatal phenotyping make it challenging for clinicians to counsel couples regarding a potential underlying genetic cause.[13] Although there are some data suggesting that CMA can increase the diagnostic yield in IUGR fetuses by 5% over G-banding karyotype,[14,15] there are limited data with regard to prenatal sequencing. Given that many fetuses with genetic conditions present with IUGR, including apparently isolated short long bones, it is important to understand the rate of monogenic disorders within this population to determine the potential value of prenatal sequencing.

Objectives

This review aimed to determine the incremental yield of prenatal sequencing over CMA or G-banding karyotype in fetuses with: (1) IUGR, or (2) short long bones in both isolated instances and instances where additional structural anomalies are identified at the prenatal sonogram.

Methods

Eligibility criteria, information sources, and search strategy

The inclusion criteria for study selection were any prospective or retrospective cohort studies and/or case series that: (1) included ≥5 cases of short long bones and/or IUGR undergoing prenatal sequencing with a clearly defined phenotype; (2) initiated testing on the basis of prenatal (as opposed to postnatal) phenotype only; (3) had a nondiagnostic CMA/karyotype result (if whole-genome sequencing was performed as an "all-in-one test" excluding copy-number variation detectable by standard CMA, this was also acceptable); and (4) had known results of the genetic testing. Cases in which sequencing was initiated postnatally were included if testing was based solely on the prenatal phenotype. Cases in which sequential Sanger sequencing for individual genes or alternative genomic testing methods (eg, methylation studies) were used were not included. For studies that were not specific to IUGR or short long bones exclusively, data regarding such cases were extracted from the paper or via author request. Only variants classified as class IV and V (ie, likely pathogenic or pathogenic) that were deemed causative of the phenotype were classified as diagnostic, with the exception of n=3 cases (autosomal dominant mental retardation 21 [n=2][16] and growth retardation, impaired intellectual development, hypotonia, and hepatopathy [n=1][17]), for which it was difficult to attribute the phenotype to the variant identified and this could have happened by chance. We included these cases because authors had included them in their analysis. All study abstracts were screened by 2 independent reviewers (F.M. and R.M.), and full manuscripts were subsequently reviewed when further information was required. Where inadequate information was

provided, the corresponding author was contacted, with the paper being rejected only if the aforementioned criteria were not met or they did not respond.

This review was performed in line with recommended methods for systematic reviews and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, and was prospectively registered on the International Prospective Register of Systematic Reviews (PROSPERO; CRD42022324680).[18,19] The following databases were searched electronically for relevant citations from January 2010 (next-generation sequencing was not an available technology before this) to April 10, 2022: MEDLINE, Embase, Web of Science, and Cochrane. Abstracts from conferences were also included if the details met the inclusion criteria. The search strategy consisted of relevant Medical Subject Headings (MeSH) terms, and keywords and word variants for "prenatal," "exome sequencing," and "abnormality" were used, with alternative terms encompassing "foetus," "foetal," "prenatal diagnosis," "antenatal," "whole exome sequencing," "exome," "whole genome sequencing," "genome, human," "sequence analysis, DNA," "anomaly," and "defect." Bibliographies of relevant articles were searched manually. The search strategy is available on request to the corresponding author, as is the study protocol.

Furthermore, also included in the analysis were 3 studies (originally selected by the search criteria above, as described in the results section) encompassing extended cohorts from: (1) the Prenatal Assessment of Genomes and Exomes study[2] (n=850 fetuses [published cohort n=596]); (2) the Columbia University Irving Medical Center study from Petrovski et al[3] (n=494 fetuses [published cohort n=234]); and (3) the BOOST Brittle Bones Before Birth (BOOSTB4) study data from Chandler et al[11] (n=40 fetuses [published cohort n=16]).

Data extraction and assessment of risk of bias

Both reviewers independently extracted data on study characteristics and outcomes. Data extracted from studies, when obtainable, included: ultrasound phenotype inclusive of features associated with IUGR secondary to placental insufficiency (eg, oligohydramnios, abnormal Doppler waveforms and low first-trimester pregnancy-associated protein-A, high beta-human chorionic gonadotropin in first or second trimesters, history of IUGR stillbirth/fetus) or phenotype more representative of a skeletal dysplasia (eg, bowed long bones, narrow thorax). IUGR cases were only included if specifically defined with fetal growth <10th centile for gestational age and having evidence of placental insufficiency as stated above. Phenotypes described in the studies as "short long bones" were included as such, with no predefined criteria because this is typically not prospectively defined within studies.[11] Where there was crossover in phenotype, F.M. and R.M. selected whether the phenotype was more suggestive of short long bones related to skeletal dysplasia or IUGR (either isolated or with additional anomalies). In the analysis, the isolated fetuses with short long bones were only included if the aforementioned criteria were assessed within the study or if a femur length-to-abdominal circumference ratio was provided to delineate the phenotypes. Short long bones with additional anomalies and with additional skeletal anomalies were recorded separately, but only those in the latter group were included in the subanalysis. In relation to additional fetal structural anomalies, subtle dysmorphology and normal variants or "soft-markers" were not included. Other parameters recorded included gestation at testing, sequencing approach, reported variants, source of fetal DNA, turnaround time (days), and pregnancy outcome.

Quality assessment was performed using modified Standards for Reporting of Diagnostic Accuracy (STARD) criteria.[20] Criteria deemed most important to optimize accuracy were: (1) trio analysis; (2) use of American College of Medical Genetics and Genomics and/or English Association for Clinical Genomic Science criteria for variant interpretation[21,22]; and (3) Sanger sequencing validation with consensus reached by F.M and R.M. Although reported, quality assessment was not used in overall final study selection before analysis because of the paucity of studies reporting on isolated IUGR and short long bones. However, for subanalyses (eg, isolated IUGR or short long bones) studies were only included if they had ≥3 cases for respective groups.

Data synthesis

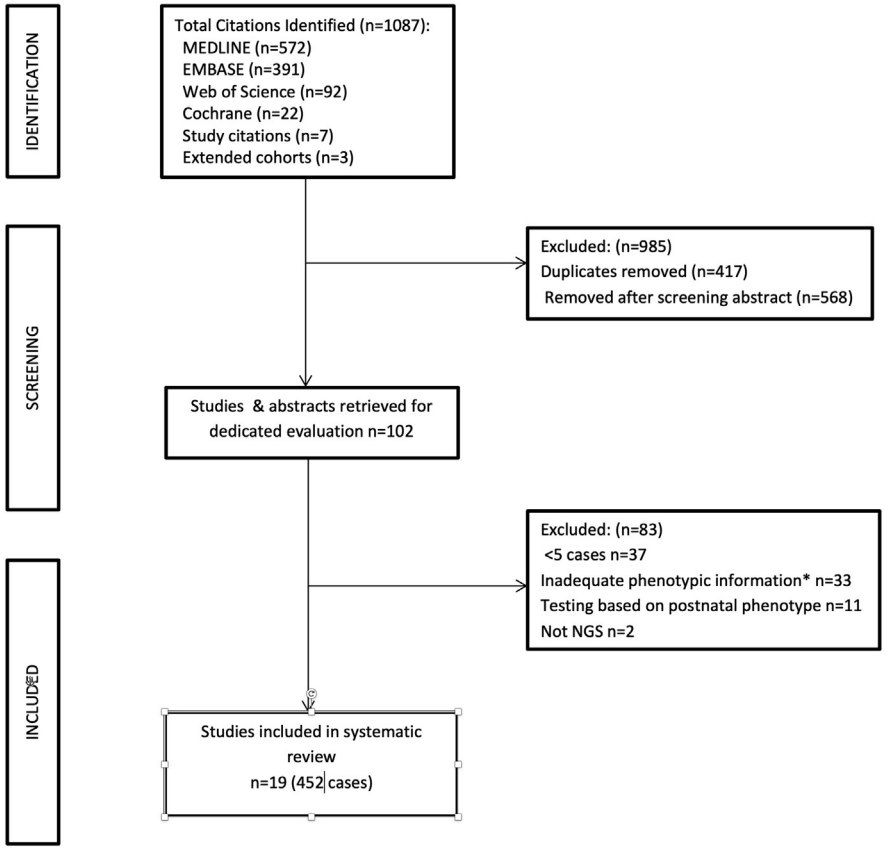
Descriptive tables were produced detailing study characteristics and outcomes. The incremental yield (or risk difference) of prenatal sequencing over CMA or karyotyping was calculated with 95% confidence intervals (CIs) for each study and as a pooled value for: (1) isolated IUGR; (2) IUGR with additional structural anomalies; (3) isolated short long bones; and (4) short long bones associated with additional skeletal features. Where reported, pooled values for variants of uncertain significance (VUS) and incidental findings (IFs) were also determined. Risk differences from each study were pooled using a random-effects model throughout to estimate incremental yield by a previously published method, which facilitated calculation with adjustment for "zero" values from negative quantitative fluorescent polymerase chain reaction and CMA or karyotype testing.[7–9] Results were displayed in forest plots with corresponding 95% CIs. Heterogeneity was assessed graphically within the forest plot and statistically using Higgins' I^2. Publication bias was assessed graphically using funnel plots. Statistical analysis was performed using RevMan version 5.3.4 (Review Manager, Cochrane Collaboration, Copenhagen, Denmark) statistical software.

Results

Study selection and characteristics

The study selection process is demonstrated in Figure 1. Where a study was suitable for inclusion but data were incomplete in relation to phenotype, the corresponding authors were contacted to request further data (n=17), of whom 4 responded. Three studies were

FIGURE 1
PRISMA flow diagram



Asterisk denotes authors contacted for further information.

NGS, next-generation sequencing.

Mone. Prenatal exome sequencing for short long bones and growth restriction. Am J Obstet Gynecol 2023.

excluded because they did not meet inclusion criteria, and 1 author provided a full dataset.[16] In addition, another n=18 studies were included (encompassing the extended datasets).[2,3,11,17,23–36] The Table highlights the characteristics of included studies, and Figure 2 shows the overall quality assessment.

Synthesis of results and risk of bias

In total, n=19 studies were included, with a total of n=452 IUGR or short long bone cases (IUGR n=116 and short long bones n=336). Of those in the IUGR group, 61.2% (n=71) were apparently isolated IUGR, and 38.8% (n=45) were cases associated with additional anomalies. In relation to short long bones, 25% (n=84) of cases were reported as isolated, and 75% (n=252) were associated with any additional fetal anomaly, of which 80.9% (n=204) had an additional skeletal anomaly and were subsequently analyzed. The mean maternal age and gestation at testing was 30.3 (±4.7 standard deviation [SD]) years and 22.6 (±4.8 SD) weeks. Where stated, fetal DNA was obtained in most cases via amniocentesis (74.3%; n=254/342). Where documented (n=13 studies), the median turnaround time for prenatal sequencing was 17 days (range, 6–56). The most common pregnancy outcome was termination of pregnancy (59.5%; n=179/301). The pooled incremental yield for VUS was 9% (95% CI, 5–14; I^2=0%; P<.001), with too few studies (n=2) reporting on IFs. A list of clinical syndromes caused by pathogenic and likely pathogenic variants included within the final meta-analysis is outlined in the Supplemental Table. Of note, 86.5% (n=32/37) of isolated short long bone cases with a diagnosis from sequencing had a skeletal dysplasia.

Systematic review of pathogenic variants

In total, there were n=224 (50%) cases in which a causative pathogenic or likely pathogenic variant was identified, of which n=199 cases were included in the subanalysis. Subgroup analysis was focused on studies in which there were >3 cases for each respective group. The apparent incremental yields with prenatal sequencing were: (1) 4% (95% CI, −5.0 to 12; I^2=0%) in isolated IUGR with evidence of placental insufficiency, (2) 30% (95% CI, 13–47; I^2=1%) in IUGR with additional anomalies, (3) 48% (95% CI, 26–70; I^2=73%) in isolated short long bones, and (4) 68% (95% CI, 58–77; I^2=51%) in short long bones with additional skeletal anomalies (Figures 3–6). Because of high levels of heterogeneity in the latter 2 groups, a random-effects model was applied. The corresponding funnel plots are displayed in Supplemental Figures 1 to 4. Where documented, the most common genetic syndromes associated with isolated short long bones were thanatophoric dysplasia and osteogenesis imperfecta, with frequencies of 21.6% (n=8/37) for each of these diagnoses. For short long bones with additional skeletal features, osteogenesis imperfecta was the most common diagnosis (28%; n=57/204) (Supplemental Table). The inheritance patterns were de novo in 75.4% (n=150/199). In instances where multisystem IUGR was associated with a pathogenic variant, the most common associated anomalies were those of the central nervous system (50%; n=7/14) and the most common ultrasound features associated with a causative variant in cases of short long bones and additional skeletal features were bowing of the long bones (55.9%; n=81/145) and a narrow thorax (44.8%; n=65/145).

Comment

Principal findings

Prenatal exome sequencing has limited incremental yield in IUGR owing to placental insufficiency, with moderate

TABLE
Characteristics of included studies

Study	NGS approach	Isolated		Multisystem		Total
		FGR	SLB	FGR	SLB	
Boissel et al,[23] 2018	WES Trio 110X coverage Agilent capture + Illumina HiSeq 2000 or 2500X	1	0	4	0	5
Chandler, et al,[11] 2018 (extended)	Clinical ES Trio N=240 skeletal dysplasia genes SureSelect Target enrichment Illumina NextSeq500	1	2	1	36	40
Daum et al,[24] 2019	WES Mainly proband only Agilent capture+ Illumina HiSeq 2500	1	0	4	4	9
Dempsey et al,[25] 2021	WES Trio NEXTSEQ 500 or NOVASEQ	2	2	1	6	11
Deden et al,[26] 2020	WES Trio 200-300 X coverage Agilent capture + Illumina NextSeq500	0	0	3	17	20
Gabriel et al,[16] 2021	WES Trio Sureselect + Agilent enrichment HiSeq4000 or the Novaseq6000 170X	22	24	3	17	66
Greenbaum et al,[27] 2019	WES Trio 100 x coverage Capture kit unknown + Illumina sequencing	2	0	2	3	7
Han et al,[28] 2020	WES trio SimbleGen SeqCap enrichment NextSeq500 2000X	0	4	0	22	26
Liu et al,[29] 2019	Targeted WES 20X 363 genes involves in dekeltal anomalies	0	8	0	5	13
Lord et al,[2] 2019 (extended)	WES Trio 1628 genes Agilent capture + Illumina Hi-Seq 2500 98.3% of the bait regions covered at a minimum depth of 5X	13	10	5	38	66
Mone et al,[30] 2022	Illumina TruSight then Illumina HiSeq 2500 n=1542 gene panel 20X and Nonacus enrichment and Illumina NextSeq550 n=1205 genes	0	0	5	9	14
Peng et al,[31] 2021	Singleton WES Agilent enrichment Novaseq 6000 platform 100X	0	5	0	25	30
Petrovski et al,[3] 2019 (extended)	WES Trio Nimblegen SeqCap EZ capture + Illumina HiSeq2500 Average coverage 89.3 reads Bioinformatic signatures	17	5	2	7	31
Rinaldi et al,[32] 2020	Trio WES Nimblegen SeqCap EZ capture and HiSeq2500	0	0	7	3	10
Tang et al,[33] 2021	Q800R Sonicator library prep. xGen Exome research panel v1.0 19,396 genesIllumina NextSeq5000	0	3	0	5	8
Zhang L et al,[34] 2021	Mainly singleton WES xGen Exome Research Panel v1.0 capture and Illumina Hiseq2000	0	17	1	18	36
Zhang X et al,[35] 2021	Trio panel (skeleton disease related panel n=505 genes) Illumina HiSeq 2000 97% coverage target regions and WES xGen Exome Research Panel v1.0 Novaseq 6000 coverage depth >20X	0	0	0	27	27
Zhou J et al,[17] 2021	WES and WGS trio 40X and 100X MGISEQ-2000	12	1	6	2	21
Zhou X et al,[36] 2021	Singleton clinical exome Agilent 2100 preparation then Illumina Hiseq 2500 97% with 20X coverage	0	3	0	9	12

ES, exome sequencing; *IUGR*, intrauterine growth restriction; *NGS*, next-generation sequencing; *SLB*, short long bones; *WES*, whole-exome sequencing; *WGS*, whole-genome sequencing.

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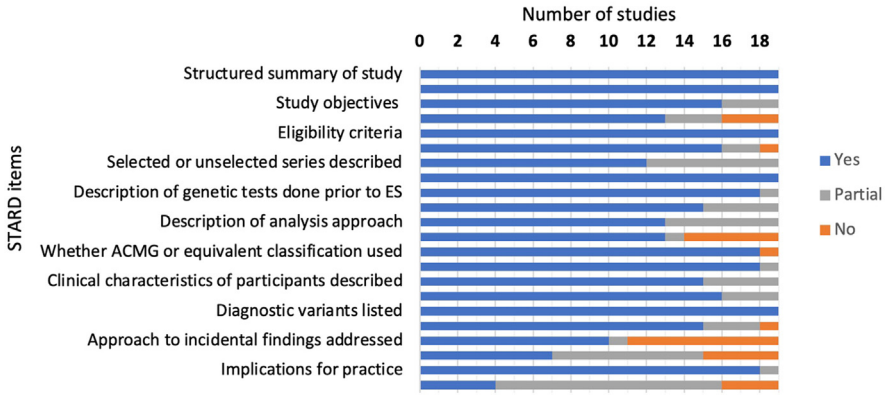
FIGURE 2
Quality assessment of studies included in systematic review



A total of 19 studies were reviewed using modified STARD criteria.

ACMG, American College of Medical Genetics and Genomics; *ES*, exome sequencing; *STARD*, Standards for Reporting of Diagnostic Accuracy.

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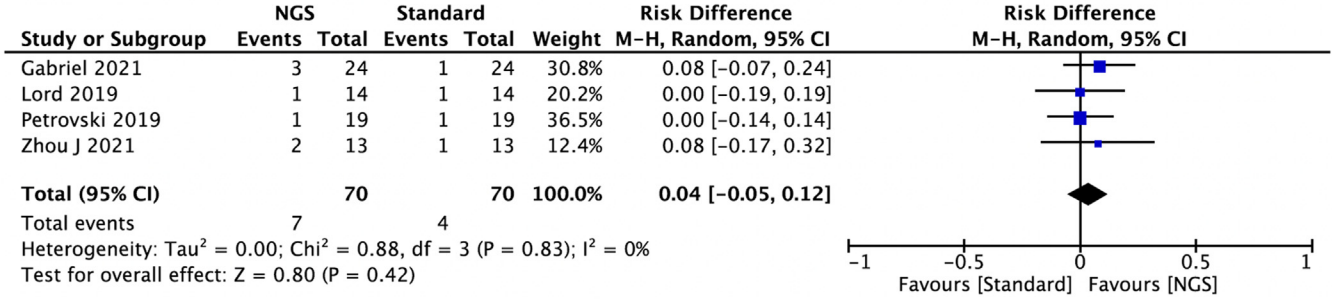
yields for IUGR associated with additional fetal structural abnormalities and isolated short long bones, and a high yield for short long bones associated with other skeletal anomalies. Most causative variants were heterozygous de novo in nature, with the most common presenting syndromes being thanatophoric dysplasia and osteogenesis imperfecta.

Comparison with existing literature

Our findings support the current evidence-based criteria for the NHS England rapid prenatal exome sequencing pathway, with the exception of isolated short long bones where there is no evidence of placental insufficiency, which may now be considered for inclusion.[6] It is not surprising that prenatal sequencing in cases of isolated IUGR has a low yield given the fact that it represents such heterogeneity in phenotype and etiology, and nonstandard classification in the literature of growth centiles, onset, Doppler parameters, and symmetry of IUGR. It is a challenge to distinguish the small-for-gestational-age fetus from the IUGR fetus, with the former often being constitutionally small.[37] Although it is proposed that early-onset IUGR is more likely to be associated with genetic etiology, there remains little support for this when there is evidence of placental insufficiency.[38] In addition, genetic origins for the multifactorial pathophysiology of this phenomenon likely lie in overlapping polymorphisms and nonmonogenic phenomena such as epigenetic modifications, uniparental disomy, and confined placental mosaicism, which prenatal sequencing cannot assess.[37] There were too few cases (n=3) of isolated IUGR within this systematic review to explore the spectrum of genetic disease in such instances.

What is also unsurprising is the high yield demonstrated for use of prenatal sequencing in short long bones with additional skeletal features. Despite being a heterogeneous group comprising individually rare conditions, skeletal dysplasias are collectively one of the most common congenital anomalies in newborns, and also represent a significant proportion of fetal anomalies detected prenatally. However, prenatal molecular diagnosis is vital, particularly if conferring "lethality." In this systematic review, many studies represented a highly selected cohort with expert phenotyping reflected by reporting long bone centiles and ratios.[11,29,31,32] It has also been identified previously that most variants in this group are de novo, likely because of their limited reproductive fitness. Exceptions to this are less frequent autosomal recessive variants, autosomal dominant variants associated with variable penetrance where a parent is unaware they have the condition, or occasionally parental mosaicism for autosomal dominant

FIGURE 3
Pooled incremental yields of pES in isolated FGR



CI, confidence interval; *pES*, prenatal exome sequencing; *IUGR*, intrauterine growth restriction; *NGS*, next-generation sequencing.

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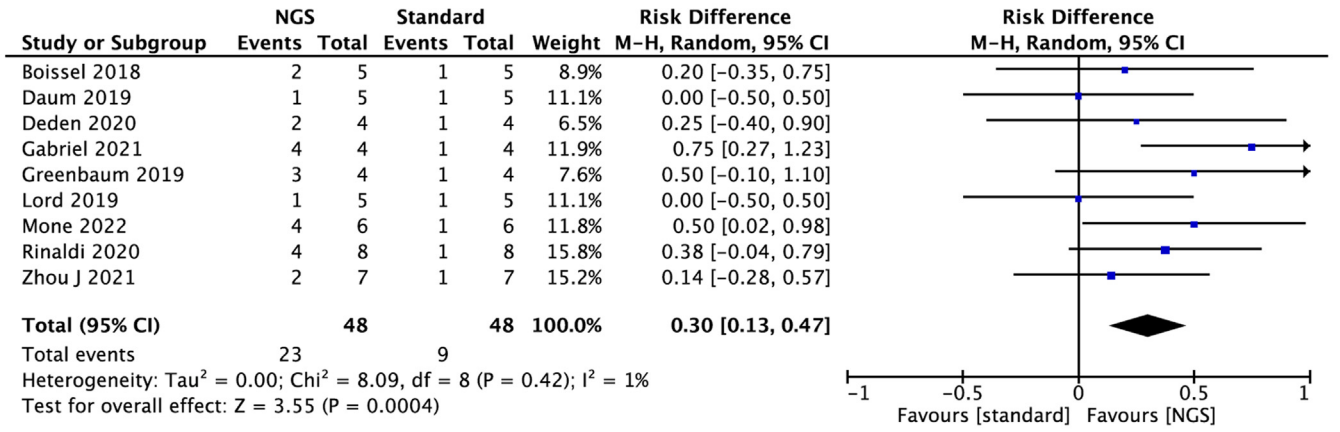
FIGURE 4
Pooled incremental yields of pES in FGR associated with additional anomalies



CI, confidence interval; *pES*, prenatal exome sequencing; *IUGR*, intrauterine growth restriction; *NGS*, next-generation sequencing.

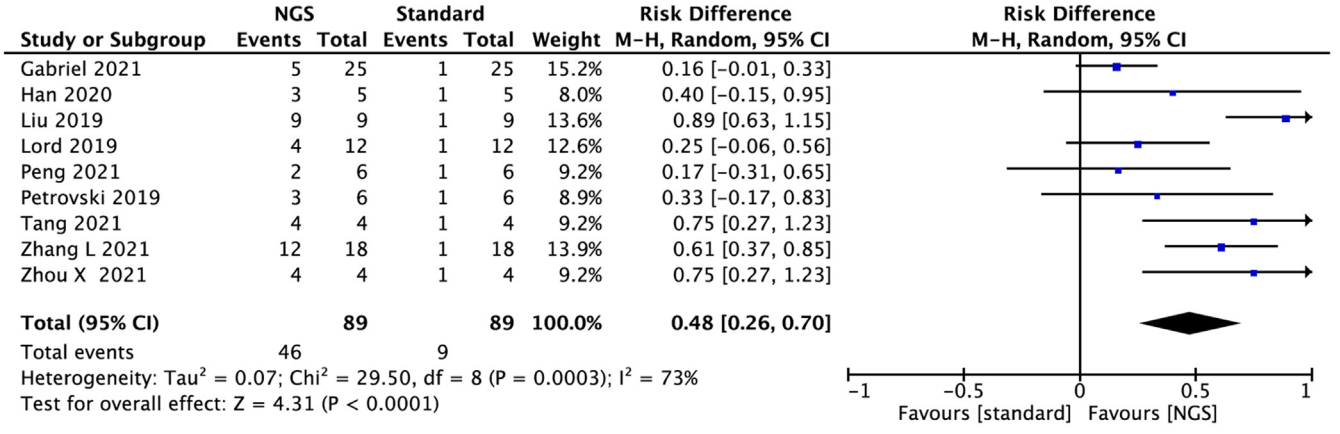
Mone. Prenatal exome sequencing for short long bones and growth restriction. Am J Obstet Gynecol 2023.

variants.[11,29,31,32] In relation to the finding of a moderate yield with isolated short long bones, most of these diagnoses were of skeletal dysplasias, conditions that would be expected to present with additional sonographic features (Supplemental Table).[39] This particularly applies to the most common skeletal dysplasias identified in our review, in which we found that thanatophoric dysplasia has features detectable from the first trimester,[40,41] the prenatal features of achondroplasia are well documented,[42] and osteogenesis imperfecta usually presents with short and bowed long bones and/or other features including a small chest and hypomineralization. Although these conditions may occasionally present as isolated short long bones, the fact that so many were defined as "isolated" highlights the need for expert ultrasonography to accurately phenotype fetuses undergoing prenatal sequencing. This would aid variant interpretation, and facilitate triage for sequencing and counseling of parents.[43]

Strengths and limitations

This was a large review assessing the provision of prenatal sequencing for IUGR or short long bones. To optimize the number of cases, corresponding authors were contacted and extended datasets were used. Despite the use of a random-effects model, heterogeneity remained relatively high, which can be explained by: (1) case selection—with some of the larger series representing unselected populations,[2,3] whereas other cohorts were highly selected as potential skeletal dysplasias[11,28,31,33,35]; and (2)

FIGURE 5
Pooled incremental yields of pES in isolated short long bones



CI, confidence interval; *pES*, prenatal exome sequencing; *NGS*, next-generation sequencing.

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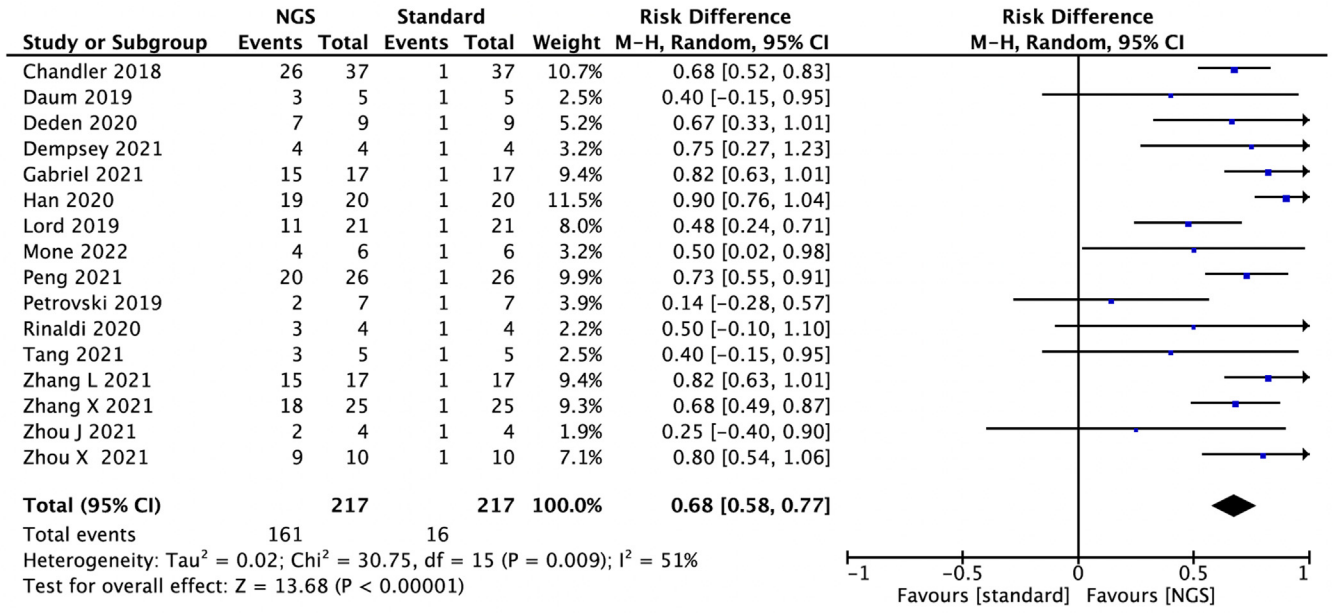
FIGURE 6
Pooled incremental yields of pES in SLB with additional skeletal features



CI, confidence interval; *pES*, prenatal exome sequencing; *NGS*, next-generation sequencing; *SLB*, short long bones.

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use of a panel or whole-exome approach with refinement of panels over time as researchers have become more familiar with this novel genomic testing strategy. The numbers of cases of isolated IUGR owing to placental insufficiency were too small to draw firm conclusions; however, this may be because it is not recognized as a true fetal anomaly and hence is not listed as part of the phenotype, or because it is recognized as being heterogeneous and not usually included in eligibility criteria for sequencing. Moving forward, a large prospective study assessing the provision of sequencing in IUGR as defined by clear criteria and exclusion of alternative etiologies is needed. What can be extrapolated clinically is limited by the nature of the data reported within the included studies; therefore, the precise definitions of isolated short long bones with the most optimal yield for prenatal sequencing cannot be deduced without further evidence. However, in the first instance it may be reasonable to restrict case selection to short long bones that are severe (eg, <third centile), early-onset (eg, <32 weeks) at presentation, and persistent over >1 episode of imaging, and then reevaluate the yield. It was presumed that where authors had defined pathogenic variants as causative that this was the case, but that it may be difficult to attribute causality to a gene where there is no obvious antenatal phenotype and there may indeed be associations that occur by chance.

Conclusions and implications

Prenatal exome sequencing has a substantial incremental yield over CMA in cases of isolated and nonisolated fetal short long bones or IUGR with multisystem abnormalities. However, our data do not support the use of prenatal sequencing in cases with isolated IUGR with evidence of placental insufficiency. Further studies are required to assess the value of prenatal sequencing in this situation. ■

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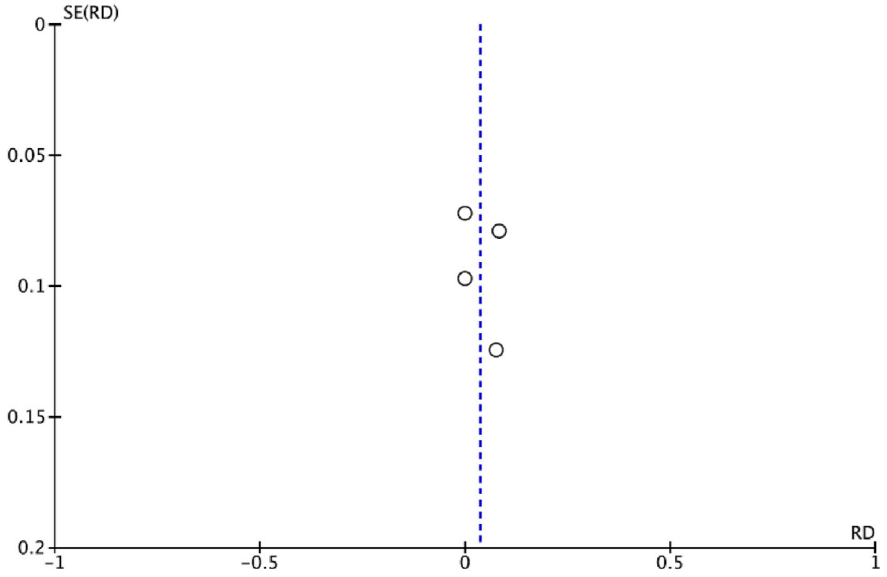
REFERENCES

1. Vora NL, Norton ME. Prenatal exome and genome sequencing for fetal structural abnormalities. Am J Obstet Gynecol 2022 [Epub ahead of print].
2. Lord J, McMullan DJ, Eberhardt RY, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. Lancet 2019;393:747–57.
3. Petrovski S, Aggarwal V, Giordano JL, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet 2019;393:758–67.
4. Mone F, McMullan DJ, Williams D, et al. Evidence to support the clinical utility of prenatal exome sequencing in evaluation of the fetus with congenital anomalies: scientific impact Paper No. 64 [February] 2021. BJOG 2021; 128:e39–50.
5. Jelin AC, Vora N. Whole exome sequencing: applications in prenatal genetics. Obstet Gynecol Clin North Am 2018;45:69–81.
6. NHS England. Rapid Exome Sequencing Service for Fetal Anomalies Testing. 2021. Available at, https://labs.gosh.nhs.uk/media/1396340/rapid_prenatal_exome_sequencing_r21_faq_v1.pdf. Accessed March 24, 2021.
7. Mone F, Eberhardt RY, Morris RK, et al. Congenital heart disease and the diagnostic yield with exome sequencing (CODE) study: prospective cohort study and systematic review. Ultrasound Obstet Gynecol 2021;57:43–51.

8. Mone F, Eberhardt RY, Hurles ME, et al. Fetal hydrops and the Incremental yield of Next-generation sequencing over standard prenatal Diagnostic testing (FIND) study: prospective cohort study and meta-analysis. Ultrasound Obstet Gynecol 2021;58:509–18.
9. Mellis R, Oprych K, Scotchman E, Hill M, Chitty LS. Diagnostic yield of exome sequencing for prenatal diagnosis of fetal structural anomalies: a systematic review and meta-analysis. Prenat Diagn 2022;42:662–85.
10. Mellis R, Eberhardt RY, Hamilton SJ, et al. Fetal exome sequencing for isolated increased nuchal translucency: should we be doing it? BJOG 2022;129:52–61.
11. Chandler N, Best S, Hayward J, et al. Rapid prenatal diagnosis using targeted exome sequencing: a cohort study to assess feasibility and potential impact on prenatal counseling and pregnancy management. Genet Med 2018;20:1430–7.
12. Weisz B, David AL, Chitty L, et al. Association of isolated short femur in the mid-trimester fetus with perinatal outcome. Ultrasound Obstet Gynecol 2008;31:512–6.
13. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Obstetrics and the Society for Maternal-Fetal Medicine. ACOG Practice Bulletin No. 204: fetal growth restriction. Obstet Gynecol 2019;133:e97–109.
14. Borrell A, Grande M, Meler E, et al. Genomic microarray in fetuses with early growth restriction: a multicenter study. Fetal Diagn Ther 2017;42:174–80.
15. Li LS, Li DZ. A genetic approach to the etiologic investigation of isolated intrauterine growth restriction. Am J Obstet Gynecol 2021;225:695–6.
16. Gabriel H, Korinth D, Ritthaler M, et al. Trio exome sequencing is highly relevant in prenatal diagnostics. Prenat Diagn 2022;42:845–51.
17. Zhou J, Yang Z, Sun J, et al. Whole genome sequencing in the evaluation of fetal structural anomalies: a parallel test with chromosomal microarray plus whole exome sequencing. Genes (Basel) 2021;12:1–14.
18. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 2009;6:e1000100.
19. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000;283:2008–12.
20. Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Standards for reporting of diagnostic accuracy. Clin Chem 2003;49:1–6.
21. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405–24.
22. Ellard S, Baple EL, Calloway A, et al. ACGS best practice guidelines for variant classification in rare disease 2020. 2021. Available at: https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf34a, . Accessed July 1, 2022.
23. Boissel S, Fallet-Bianco C, Chitayat D, et al. Genomic study of severe fetal anomalies and discovery of GREB1L mutations in renal agenesis. Genet Med 2018;20:745–53.
24. Daum H, Meiner V, Elpeleg O, Harel T; collaborating authors. Fetal exome sequencing: yield and limitations in a tertiary referral center. Ultrasound Obstet Gynecol 2019;53:80–6.
25. Dempsey E, Haworth A, Ive L, et al. A report on the impact of rapid prenatal exome sequencing on the clinical management of 52 ongoing pregnancies: a retrospective review. BJOG 2021;128:1012–9.
26. Deden C, Neveling K, Zafeiropopoulou D, et al. Rapid whole exome sequencing in pregnancies to identify the underlying genetic cause in fetuses with congenital anomalies detected by ultrasound imaging. Prenat Diagn 2020;40:972–83.
27. Greenbaum L, Pode-Shakked B, Eisenberg-Barzilai S, et al. Evaluation of diagnostic yield in fetal whole-exome sequencing: a report on 45 consecutive families. Front Genet 2019;10:425.
28. Han J, Yang YD, He Y, et al. Rapid prenatal diagnosis of skeletal dysplasia using medical trio exome sequencing: benefit for prenatal counseling and pregnancy management. Prenat Diagn 2020;40:577–84.
29. Liu Y, Wang L, Yang YK, et al. Prenatal diagnosis of fetal skeletal dysplasia using targeted next-generation sequencing: an analysis of 30 cases. Diagn Pathol 2019;14:76.
30. Mone F, Abu Subieh H, Doyle S, et al. Evolving fetal phenotypes and clinical impact of progressive prenatal exome sequencing pathways: cohort study. Ultrasound Obstet Gynecol 2022;59:723–30.
31. Peng Y, Yang S, Huang X, et al. Whole exome sequencing analysis in fetal skeletal dysplasia detected by ultrasonography: an analysis of 38 cases. Front Genet 2021;12:728544.
32. Rinaldi B, Race V, Corveleyn A, et al. Next-generation sequencing in prenatal setting: some examples of unexpected variant association. Eur J Med Genet 2020;63:103875.
33. Tang H, Zhang Q, Xiang J, Yin L, Wang J, Wang T. Whole exome sequencing aids the diagnosis of fetal skeletal dysplasia. Front Genet 2021;12:599863.
34. Zhang L, Pan L, Teng Y, Liang D, Li Z, Wu L. Molecular diagnosis for 55 fetuses with skeletal dysplasias by whole-exome sequencing: a retrospective cohort study. Clin Genet 2021;100:219–26.
35. Zhang X, Ren Y, Song R, et al. Combined exome sequencing and deep phenotyping in highly selected foetuses with skeletal dysplasia during the first and second trimesters improves diagnostic yield. Prenat Diagn 2021;12:1401–13.
36. Zhou X, Zhou J, Wei X, et al. Value of exome sequencing in diagnosis and management of recurrent non-immune hydrops fetalis: a retrospective analysis. Front Genet 2021;12:616392.
37. Nowakowska BA, Pankiewicz K, Nowacka U, Niemiec M, Kozłowski S, Issat T. Genetic background of fetal growth restriction. Int J Mol Sci 2021;23:36.
38. Meler E, Sisterna S, Borrell A. Genetic syndromes associated with isolated fetal growth restriction. Prenat Diagn 2020;40:432–46.
39. Chandler NJ, Scotchman E, Mellis R, Ramachandran V, Roberts R, Chitty LS. Lessons learnt from prenatal exome sequencing. Prenat Diagn 2022;42:831–44.
40. Khalil A, Pajkrt E, Chitty LS. Early prenatal diagnosis of skeletal anomalies. Prenat Diagn 2011;31:115–24.
41. Chitty LS, Khalil A, Barrett AN, Pajkrt E, Griffin DR, Cole TJ. Safe, accurate, prenatal diagnosis of thanatophoric dysplasia using ultrasound and free fetal DNA. Prenat Diagn 2013;33:416–23.
42. Chitty LS, Griffin DR, Meaney C, et al. New aids for the non-invasive prenatal diagnosis of achondroplasia: dysmorphic features, charts of fetal size and molecular confirmation using cell-free fetal DNA in maternal plasma. Ultrasound Obstet Gynecol 2011;37:283–9.
43. Gray KJ, Wilkins-Haug LE, Herrig NJ, Vora NL. Fetal phenotypes emerge as genetic technologies become robust. Prenat Diagn 2019;39:811–7.

SUPPLEMENTAL FIGURE 1
Funnel plot of isolated fetal growth restriction



RD, risk difference; *SE*, standard error.

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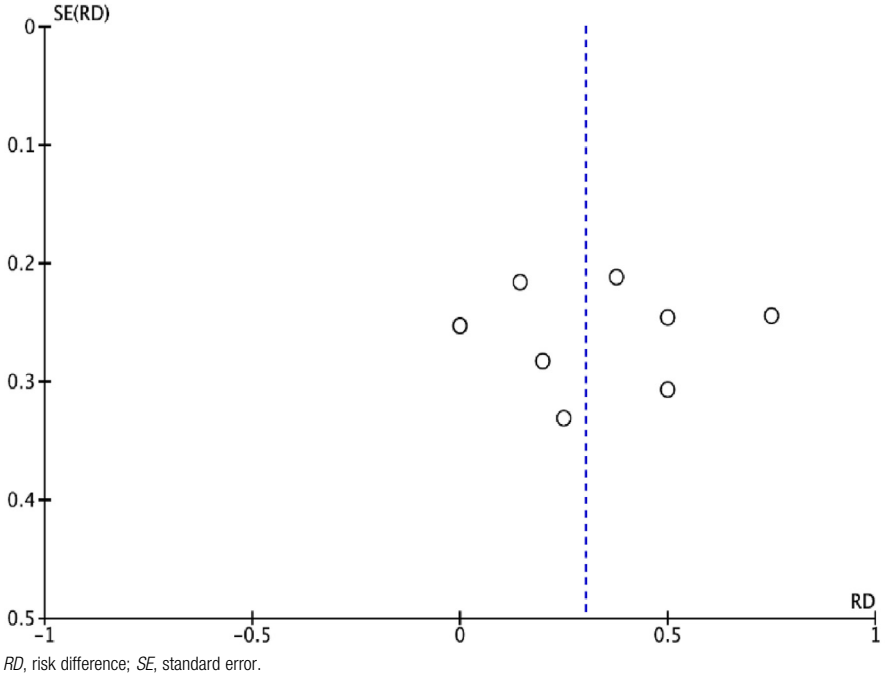
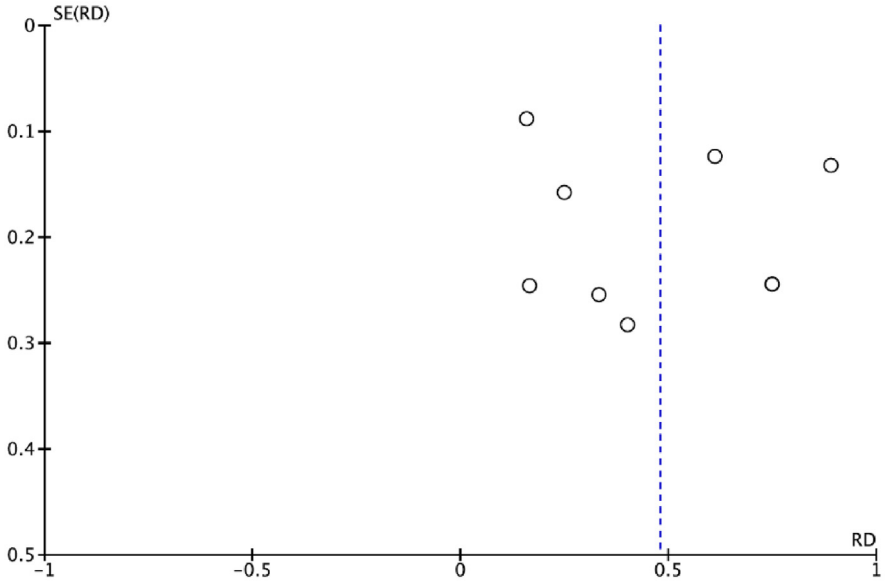
SUPPLEMENTAL FIGURE 2
Funnel plot of multisystem fetal growth restriction



RD, risk difference; *SE*, standard error.

Mone. Prenatal exome sequencing for short long bones and growth restriction. Am J Obstet Gynecol 2023.

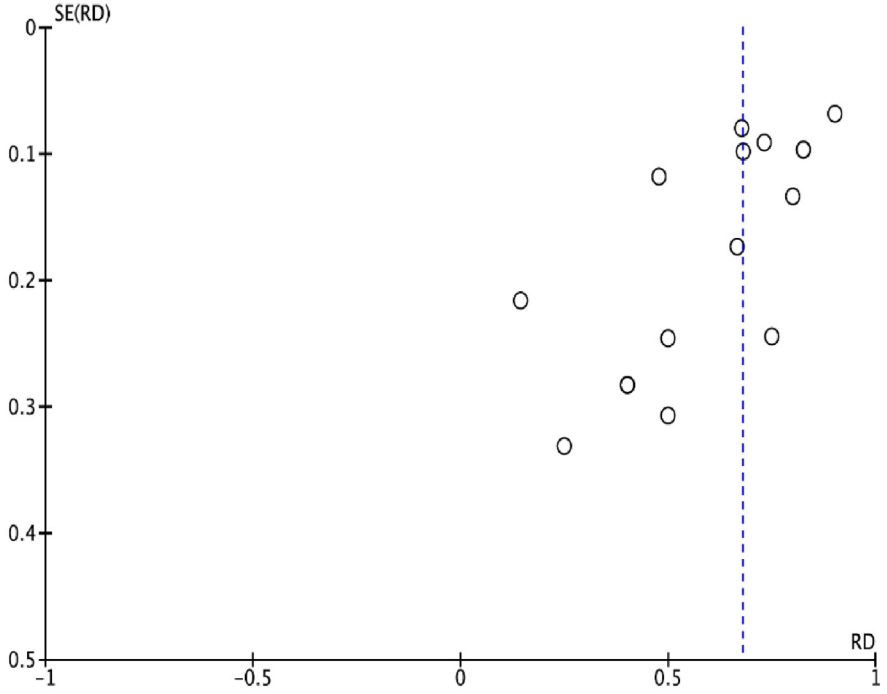
SUPPLEMENTAL FIGURE 3
Funnel plot of isolated short long bones



RD, risk difference; *SE*, standard error.

Mone. Prenatal exome sequencing for short long bones and growth restriction. Am J Obstet Gynecol 2023.

SUPPLEMENTAL FIGURE 4
Funnel plot of short long bones with additional skeletal features



RD, risk difference; *SE*, standard error.

Mone. Prenatal exome sequencing for short long bones and growth restriction. Am J Obstet Gynecol 2023.

SUPPLEMENTAL TABLE

List of clinical syndromes included in meta-analysis

Clinical syndrome	Isolated FGR	Multisystem FGR	Isolated SLB	SLB and additional skeletal anomalies	Total
Osteogenesis imperfecta			8	57	65
Thanatophoric dysplasia			8	24	32
Achondroplasia			5	15	20
Achondrogenesis type II			1	7	8
Short-rib polydactyly			1	7	8
Hypochondroplasia			5	1	6
Chondrodysplasia punctata			1	3	4
Spondylo-peripheral dysplasia				3	3
Congenital disorder of glycosylation			1	2	3
Otospondylomegaepiphyseal dysplasia				2	2
Diastrophic dysplasia				2	2
Shwachman–Diamond syndrome		1	1		2
Autosomal dominant mental retardation	1	1			2
Campomelic dysplasia				2	2
Collagen, type II				2	2
Greenberg skeletal dysplasia				2	2
3-M syndrome 1			1	1	2
Kniest dysplasia			1		1
Ellis–van Creveld syndrome				1	1
Thrombocytopenia-absent radius syndrome				1	1
Kabuki syndrome		1			1
CHARGE syndrome		1			1
Nijmegen breakage syndrome		1			1
Neu–Laxova syndrome				1	1
Dyserythropoietic anemia, congenital type II		1			1
Microcephaly, short stature, and polymicrogyria with seizures		1			1
Arthrogryposis, distal, type 5D		1			1
Brachydactyly type A1			1		1
Osteochondrodysplasia, complex lethal, Symoens-Barnes-Gistelinck type				1	1
Cutis laxa, autosomal recessive type IIB o				1	1
Epiphyseal dysplasia, multiple, 4			1		1
Hypophosphatasia				1	1
Spondyloepiphyseal dysplasia congenita				1	1
Netherton syndrome	1				1
Diamond–Blackfan-anemia 1		1			1
Menkes disease		1			1
Stickler syndrome, type II			1		1
Stüve–Wiedemann syndrome			1		1

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(continued)

SUPPLEMENTAL TABLE

List of clinical syndromes included in meta-analysis *(continued)*

Clinical syndrome	Isolated FGR	Multisystem FGR	Isolated SLB	SLB and additional skeletal anomalies	Total
SADDAN dysplasia				1	1
Marshall syndrome				1	1
Bent bone dysplasia syndrome				1	1
Rubinstein–Taybi syndrome 2		1			1
Myasthenic syndrome, congenital, 5				1	1
Growth retardation, impaired intellectual development, hypotonia, and hepatopathy	1				1
Gracile bone dysplasia				1	1
Mucopolysaccharidosis type II				1	1
Cerebro-oculo-facio-skeletal syndrome 3		1			1
Cleidocranial dysplasia				1	1
Cornelia de Lange syndrome				1	1
Joubert syndrome		1			1
Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies, type A, 11)		1			1
Total	3	14	37	145	199

FGR, fetal growth restriction; *SADDAN*, severe achondroplasia with developmental delay and acanthosis nigricans; *SLB*, short long bones.

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