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Diagnostic yield of exome sequencing in fetuses with multisystem malformations: systematic review and meta-analysis

M. PAUTA[1], R. J. MARTINEZ-PORTILLA[2] and A. BORRELL[1,3]

[1]*BCNatal, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Catalonia, Spain;* [2]*Clinical Research Division, Evidence-Based Medicine Department, National Institute of Perinatology, Mexico City, Mexico;* [3]*Barcelona Centre for Maternal-Fetal and Neonatal Medicine (BCNatal), Hospital Clínic Barcelona, Universitat de Barcelona, Barcelona, Catalonia, Spain*

KEYWORDS: diagnostic yield; exome sequencing; fetal structural anomaly; multisystem anomalies; prenatal diagnosis

CONTRIBUTION

What are the novel findings of this work?
Our study provides the first systematic review and meta-analysis of evidence on the diagnostic yield of exome sequencing in fetuses with multisystem anomalies. It revealed that exome-sequencing analysis provides a 33% incremental yield in fetuses with multisystem malformations and normal chromosome analysis.

What are the clinical implications of this work?
Exome sequencing should be considered when prenatal multisystem anomalies are observed.

ABSTRACT

Objective *To determine the diagnostic yield of exome sequencing (ES) above that of chromosomal microarray analysis (CMA) or karyotyping in fetuses with multisystem structural anomalies (at least two major anomalies in different anatomical systems).*

Method *This was a systematic review conducted in accordance with PRISMA guidelines. Searching PubMed, Web of Knowledge and Cochrane database, we identified studies describing ES, whole-genome and/or next-generation sequencing in fetuses with multisystem malformations. Included were observational studies involving five or more eligible fetuses. A fetus was eligible for inclusion if it had at least two major anomalies of different anatomical systems and a 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. The diagnostic yield of the primary outcome was calculated by single-proportion analysis using random-effects modeling. A subgroup analysis was performed to compare the diagnostic yield of the solo approach (fetus alone sequenced) with that of the trio approach (fetus and both parents sequenced).*

Results *Seventeen articles with data on ES diagnostic yield, including 694 individuals with multisystem malformations, were identified. Overall, a pathogenic or likely pathogenic variant potentially causative of the fetal phenotype was found in 213 fetuses, giving a 33% (95% CI, 27–40%) incremental yield of ES. A stratified analysis showed similar diagnostic yields of ES using the solo approach (30%; 95% CI, 11–52%) and the trio approach (35%; 95% CI, 26–44%).*

Conclusions *ES applied in fetuses with multisystem structural anomalies was able to identify a potentially causative gene when CMA or karyotyping had failed to do so in an additional one-third of cases. No differences were observed between the solo and trio approaches for ES.* © *2022 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Fetal structural anomalies occur in approximately 2.5% of pregnancies[1] and most of them can be identified by ultrasound examination. When a fetal structural anomaly is detected, normally, chromosomal microarray analysis (CMA) is offered after amniocentesis, chorionic villus sampling or fetal blood sampling. CMA may reveal 6–10% of pathogenic microdeletions or microduplica-

Correspondence to: Dr A. Borrell, BCNatal, Seu Maternitat de l'Hospital Clínic, Sabino Arana 1, Barcelona 08028, Catalonia, Spain (e-mail: aborrell@clinic.cat)

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tions in addition to the 14% of chromosomal anomalies detectable by karyotyping[2,3].

Exome-sequencing (ES) enables assessment of the coding regions of more than 20 000 genes. Although it covers approximately only 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[4,5], and nowadays it is being applied increasingly in fetuses with structural anomalies and normal CMA results[6]. To simplify its interpretation and minimize inconclusive findings, ES can be restricted to the analysis of coding sequences of the Online Mendelian Inheritance in Man (OMIM) genes only (clinical or medical ES), or to genes described previously in association with a specific condition (gene panels). 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.

A rapid scoping review published recently on ES in the prenatal setting showed that the use of prenatal ES is clearly an emerging field[7]. The diagnostic yield achieved by prenatal ES varies across studies and, often, yields cannot be combined into an overall diagnostic yield because of discrepancies among publications in the eligibility criteria and in the phenotypes of the affected fetuses[7]. Currently, the rate of monogenic disorders that have been revealed to be causative of fetal structural anomalies appears to be about 10%. However, the frequency is higher in cases of multisystem malformations, that is, at least two major anomalies in different anatomical systems, as would be expected in a syndromic fetus.

In this study, we performed a systematic review of the literature and meta-analysis to assess the diagnostic yield of ES in fetuses with multisystem malformations and a negative result on CMA or karyotyping.

METHODS

Protocol and registration

The study protocol was registered prospectively on PROPERO (International Prospective Register of Systematic Reviews) (CRD42021250918). There is no requirement 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[8]. The protocol was agreed among the authors before running the analysis, and one of them (R.J.M.P.), being external to the group, acted as a reviewer.

Eligibility criteria

Included in this systematic review were observational studies involving at least five eligible fetuses. A fetus was eligible for inclusion if it met the following criteria: at least two major anomalies of different anatomical systems and a 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, therefore variants of uncertain significance (VOUS) and secondary findings were not extracted. Both ES and genome sequencing can be applied using a solo (the fetus alone is sequenced) or a trio (both parents and fetus are sequenced) approach, and all approaches were included. The following studies were excluded: case reports; opinion articles or letters; those in which gene panels were applied; those with cases with an identified disease in the family history; and those 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, the Web of Knowledge and the Cochrane database 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. The first search, a scoping review, was carried out on 15 April 2021. This was then updated with a second search, an iterative review, on 20 June 2021. Details of the searches and the MeSH terms used are given in Figure 1.

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. In cases of relevant studies with missing information, the corresponding authors were contacted by e-mail.

Data collection process and data items

The following data were extracted onto a datasheet based on a Cochrane Consumers and Communication Review Group data extraction template: site and country in which the study was carried out, study period, study inclusion criteria, sample size, number of fetuses with multisystem malformations, interpretation approach (whole/clinical ES, solo/trio approach), Sanger sequencing validation, criteria for variant classification, and ES results and positive diagnoses.

Outcome measures

The primary outcome was the diagnostic yield of ES among fetuses with multisystem malformations (at least two major anomalies from different anatomical systems) and normal chromosome as assessed by karyotyping or CMA.

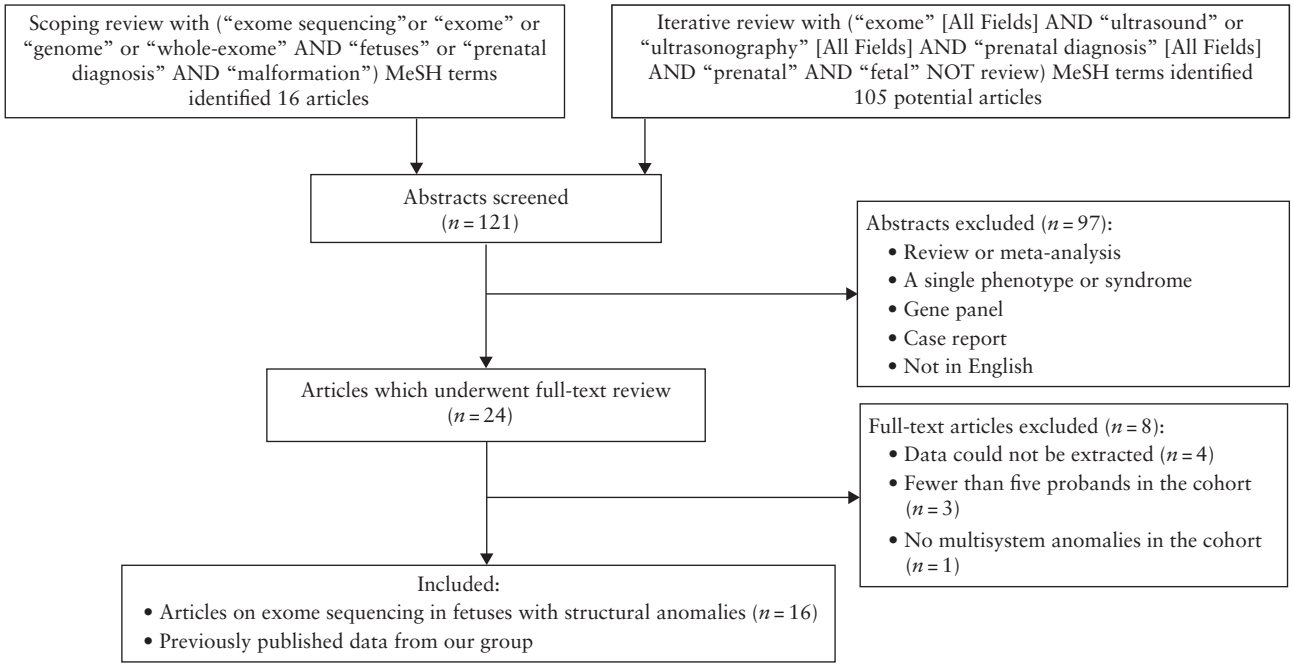

Figure 1 Flowchart summarizing inclusion in systematic review of studies reporting on diagnostic yield of exome sequencing in fetuses with multisystem malformations with a negative chromosome analysis (chromosomal microarray analysis and/or karyotyping) and no family history.

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) well-defined inclusion criteria; (c) study including only fetuses with multisystem structural anomalies; (d) prospective series; (e) consecutive cases; (f) ES analysis in trio (both parents and fetus); (g) the same previous genetic test (i.e. CMA/karyotyping or quantitative fluorescent polymerase chain reaction) used in the whole series; (h) VOUS and/or incidental findings reported; (i) Sanger sequencing validation; (j) variants classified according to the American College of Medical Genetics and Genomics (ACMG) criteria[9]. 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 of ES was calculated by single-proportion analysis using random-effects modeling (weighted by inverse of variance), along with the Clopper–Pearson exact method for calculation of confidence intervals[10,11]. In the event of a small number of studies (i.e. fewer than three), a fixed-effects model was preferred. Between-study heterogeneity/variability was assessed using the tau^2, χ^2 (Cochrane Q) and I^2 statistics. Results were assessed using forest plots and presented as proportions. Publication bias was assessed visually using funnel plots[12,13], quantified by the Egger method (weighted linear regression of the treatment effect on its standard error)[14] and adjusted using the Copas model for selection bias[15,16]. Statistical analyses were conducted using R studio v1.0.136 (The R Foundation for Statistical Computing; meta v4.2 package[17]).

RESULTS

For the scoping review selection process, 16 studies were selected initially from PubMed, which focused on ES and multisystem malformations. During the iterative review we found 105 series of structurally abnormal fetuses that included fetuses with multisystem malformations (Figure 1). Among the 121 abstracts found at that search, 24 articles were reviewed fully, of which 16 were deemed eligible for inclusion in the study[4,5,18–31]. Finally, our own series[32] was added. Among the 17 studies included in this systematic review, there were seven in which whole ES was performed, eight series in which only the OMIM genes were studied and two series in which the interpretation approach was not specified. Thirteen series studied both proband and parental samples (trio-ES), two studied only proband samples (solo-ES) and in the remaining two studies this was not specified (Table 1). Six studies were conducted in the USA, four in The Netherlands, two in the UK, and one in each of the following countries: Switzerland, China, Israel and Denmark, as well as our own data from Catalonia, Spain (Table 1). The study sample sizes ranged from five to 143 fetuses with multisystem malformations. The quality of the studies was found to be high after applying the modified STARD criteria (Figure 2).

The 17 selected series included 694 cases, of which 213 had a positive diagnosis on ES. The rate of pathogenic or likely pathogenic variants among fetuses with multisystem anomalies and normal chromosomes, according to the random-effects model, was 33% (95% CI, 27–40%) (Figure 3). The diagnostic yield observed in each of the 17 studies included ranged from 15% to 71%, being 15% and 38% in the two studies that had more than 100 cases[4,21]. Publication bias, assessed by linear regression asymmetry test, showed no significant quantification of bias (bias: 1.75; $P = 0.675$), as depicted empirically by the funnel plot (Figure S1). The diagnostic yield was not significantly different between studies using the trio-ES approach (35%; 95% CI, 26–44%) and those using the solo-ES approach (31%; 95% CI, 23–39%) (Figure S2).

Regarding the description of structural anomalies in individual fetuses, this was lacking in two studies[4,30], while in three studies it was provided only for positive cases[5,22,31]. Among the remaining 12 studies[18–21,23–29,32], the mean number of systems involved was 2.8 (1079/383) per fetus (Table S1). The highest proportion of anomalies was observed for the musculoskeletal system (19%) and the central nervous system (18%), while the lowest proportions were observed among respiratory anomalies (3%) and fetal hydrops (3%) (Table S1). In relation to the diagnostic yield of individual anatomical systems involved in cases with multisystem anomalies, the highest yields were observed for craniofacial dysmorphism (47% of fetuses with this anomaly were positive on ES), central nervous system anomalies (45%) and musculoskeletal

Table 1 Characteristics of the 17 studies included in this systematic review and meta-analysis

Study	*Site*	*Fetuses with multisystem anomalies* (n)	*Only multisystem anomalies*	*Interpretation approach*	*Sanger validation*	*ACMG criteria*	*Positive diagnosis on ES* (n)
Carss (2014)[18]	Birmingham, UK	12	No	Trio-WES	No	No	3
Alamillo (2015)[19]	Aliso Viejo, CA, USA	5	No	Trio-WES	No	No	3
Yates (2017)[20]	Gaithersburg, MD, USA	52	No	Solo-CES	Yes	Yes	10
Normand (2018)[21]	Houston, TX, USA	104	No	NS	Yes	Yes	39
Fu (2018)[22]	Guangzhou, China	39	No	Trio-WES	Yes	Yes	12
Aarabi (2018)[23]	Pittsburgh, PA, USA	8	No	Trio-WES	No	Yes	2
Meier (2019)[24]	Basel, Switzerland	16	No	Trio-WES	Yes	Yes	7
Lord (2019)[4]	Hinxton, UK	143	No	Trio-CES	Yes	Yes	22
Petrovski (2019)[5]	New York, NY, USA	23	No	Trio-WES	Yes	Yes	8
Daum (2019)[25]	Jerusalem, Israel	22	No	NS	—	—	6
de Koning (2019)[26]	Leiden, The Netherlands	14	No	Trio-CES	No	Yes	10
Corsten-Janssen (2020)[27]	Groningen, The Netherlands	11	No	Trio-CES	Yes	No	4
Deden (2020)[28]	Nijmegen, The Netherlands	17	No	Trio-CES	Yes	No	4
Vora (2020)[29]	Chapell Hill, NC, USA	53	No	Trio-CES	Yes	No	20
Becher (2020)[30]	Aarhus, Denmark	13	No	Trio-CES	No	Yes	8
Diderich (2021)[31]	Rotterdam, The Netherlands	93	No	Trio-WES	Yes	Yes	27
Pauta (2021)[32]	Barcelona, Catalonia, Spain	69	No	Solo-CES	Yes	Yes	28

Only first author of each study is given. ACMG, American College of Medical Genetics and Genomics; CES, clinical-exome sequencing (exome sequencing restricted to analysis of coding sequences of the OMIM genes); ES, exome sequencing; NS, not stated; WES, whole-exome sequencing.

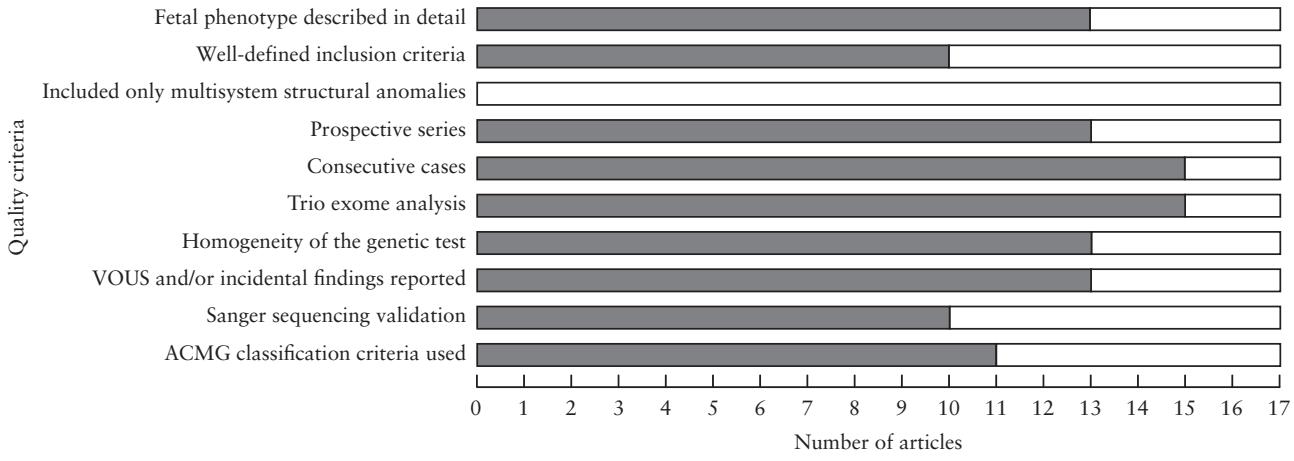

Figure 2 Quality assessment of the 17 studies included in this systematic review. ACMG, American College of Medical Genetics and Genomics; VOUS, variant of unknown significance.

anomalies (43%), while the lowest yields were for fetal hydrops (19%) and gastrointestinal anomalies (27%) (Figure 4 and Table S1). Among the 17 selected studies, only three reported data on incidental findings[22,31,32], resulting in a frequency ranging from 1.1% to 6.1%, while four reported on secondary findings[4,26,27,29], with a frequency ranging from 3.9% to 21%.

Among the 213 cases with a positive molecular diagnosis on ES, 223 genes were found to be involved, with more than one gene being involved in 10 positive cases. The genes most frequently affected were the following: the *KMT2D* gene, associated with Kabuki syndrome ($n = 11$), the *CHD7* gene, related to CHARGE syndrome ($n = 7$), the *FGFR2*-related

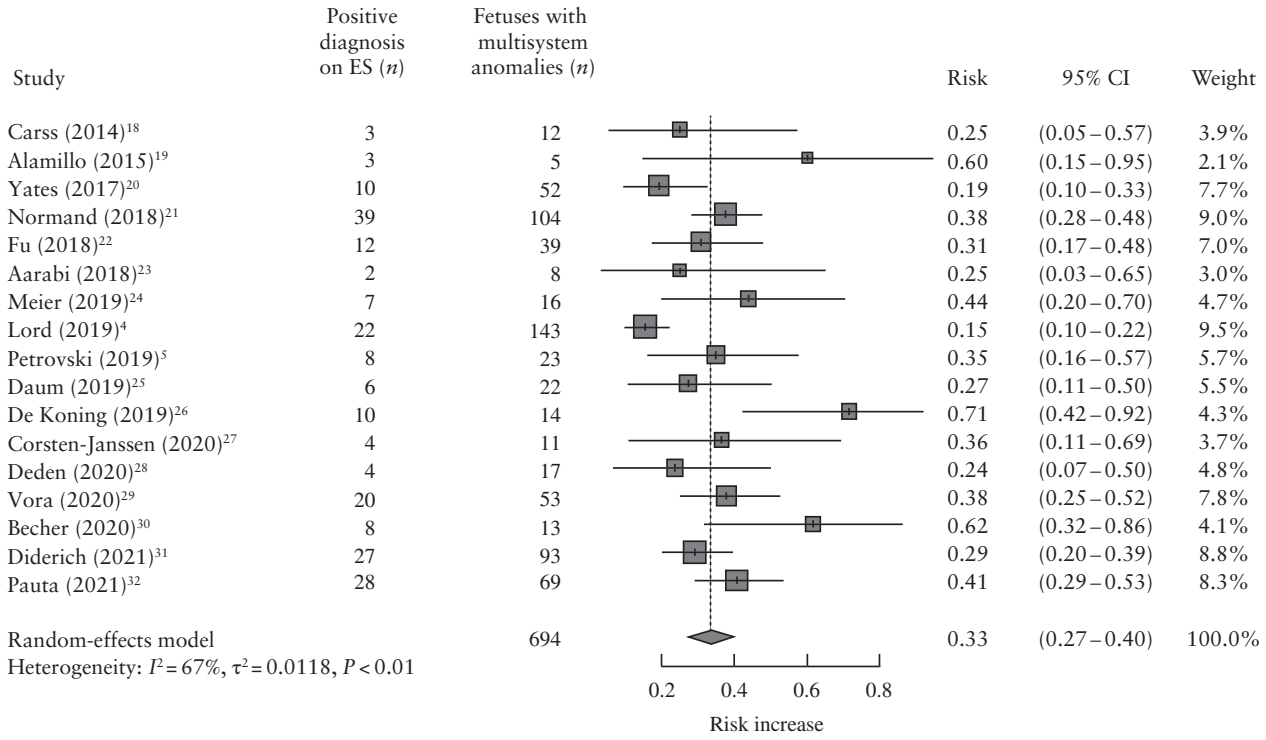

Figure 3 Forest plot of the diagnostic yield of exome sequencing (ES) in 17 studies including 694 fetuses with multisystem structural anomalies. Only first author of each study is given.

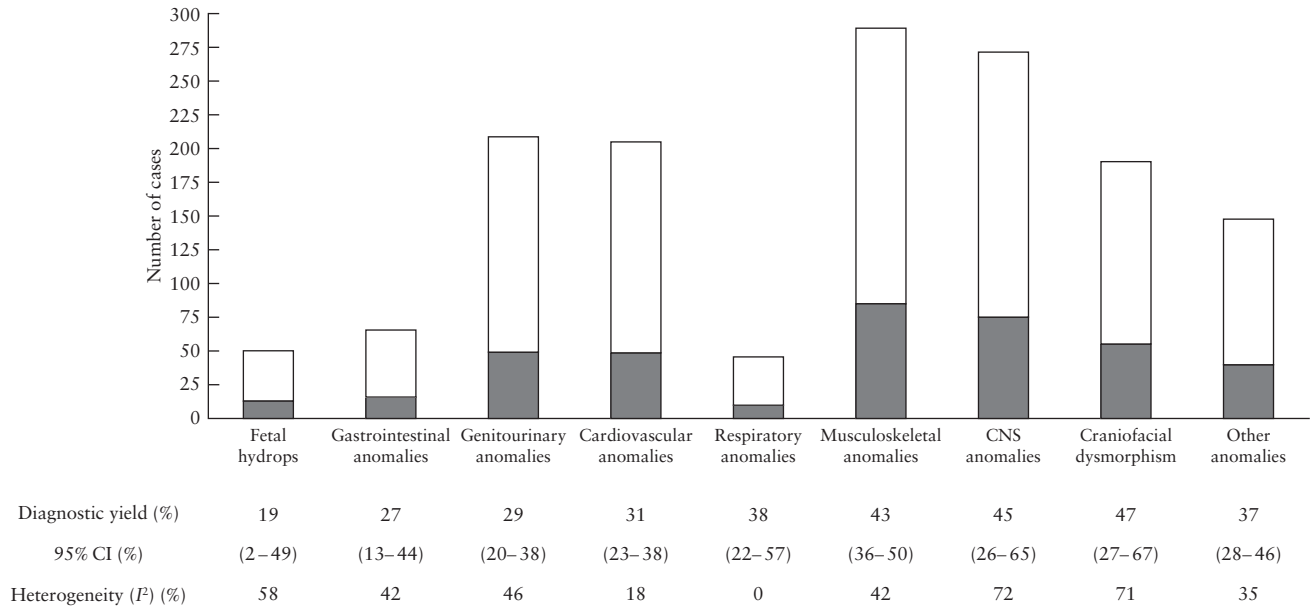

Figure 4 Diagnostic yield of exome sequencing (ES) according to individual anatomical systems or anomaly types, in fetuses with multisystem structural anomalies. ■, Conclusive cases; □, inconclusive cases. CNS, central nervous system.

disorders gene ($n=5$) and the *DHCR7* gene, related to Smith-Lemli-Opitz syndrome ($n=5$). A gene involved in the group of RASopathy syndromes was found in 22 positive cases, specifically the following genes: *PTPN11* ($n=5$), *RIT1* ($n=4$), *HRAS* ($n=4$), *KRAS* ($n=2$), *SOS1* ($n=2$), *RAF1* ($n=2$), *BRAF* ($n=2$) and NRAS ($n=1$). The second group of genes found most frequently included those related to the correct formation of collagen structures. These were found in 12 cases, four of which were associated with the *COL1A1* gene, involved in Caffey disease and osteogenesis imperfecta type I. These syndromes tended to be associated with patterns of anomalies, as follows: Kabuki syndrome with cardiac defect, often associated with a renal anomaly; CHARGE syndrome with cardiac and craniofacial defects; *FGFR2*-related diseases with musculoskeletal and craniofacial anomalies; Smith-Lemli-Opitz syndrome with skeletal anomalies, associated with cardiac or genitourinary anomalies or growth restriction; RASopathies with hydrops and additional defects; and the collagen group with musculoskeletal anomalies and hydrops.

DISCUSSION

This systematic review and meta-analysis of ES in anomalous fetuses with multisystem structural anomalies and normal chromosomes on CMA or karyotyping revealed a 33% (95% CI, 27–40%) diagnostic yield. It is well established that the risk of chromosomal anomalies on karyotyping and pathogenic copy number variants on CMA is higher in fetuses with multiple anomalies than in those with single anomalies, and also in those with multisystem multiple anomalies than in those with single-system multiple anomalies. The increase in diagnostic yield by applying ES in fetuses with multisystem anomalies should further aid the decision-making of parents of affected fetuses, adding relevant information about intellectual and neurodevelopmental expectations to the observed structural anomalies.

A higher yield in fetuses with abnormalities affecting multiple organ systems is a common finding of reported ES series. Normand *et al.*[21], in an early series of suspected Mendelian disorders with a high overall diagnostic yield (32%), showed a higher molecular diagnostic rate in fetuses with multisystem anomalies (38%) than in those with abnormalities in a single organ system (17%). Vora *et al.*[29] also found that the highest ES yield (38%) was observed in fetuses with multiple anomalies. In another large series, Diderich *et al.*[31] found that the diagnostic yield increased consistently, from 4.8% in fetuses with soft markers, to 17% in those with apparently isolated single-system malformations, 23% in those with multiple anomalies in a single system and 29% in those with multisystem anomalies.

Early guidelines recommended the use of ES in the prenatal setting only in selected fetal anomalies suggestive of a genetic disorder when genetic tests specific for the phenotype had failed to determine a diagnosis. Hence, a 2012 guideline by the ACMG[33] recommended ES for fetuses with multiple congenital anomalies with no diagnosis on standard testing and the Committee on Genetics and the Society for Maternal-Fetal Medicine[34], in 2016, recommended it for recurrent fetal phenotypes. 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[35]. A recent joint-position statement, 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[36].

There are some limitations of the evidence in this systematic review. One of the main drawbacks of prenatal ES is that prenatal phenotypes differ considerably from postnatal descriptions of the same syndrome, but, currently, ES is expanding the knowledge of prenatal presentations of various disorders. The two most common syndromes revealed by ES in fetuses with multisystem anomalies in this systematic review were CHARGE and Kabuki syndromes, both related to genes that function in epigenetic regulation (epigenes). Kabuki syndrome is characterized postnatally by typical facial features, minor skeletal anomalies, persistence of fetal fingertip pads, mild-to-moderate intellectual disability and postnatal growth deficiency. However, prenatally, it is associated with several phenotypes, including multisystem anomalies, isolated complex cardiac defects and fetal hydrops and cystic hygroma[4]. In our review, all such cases presented with cardiac defects associated with other anomalies, such as renal anomalies, growth restriction or hydrops. Another potential limitation of systematic reviews lies in discrepancies in the eligibility criteria among publications and the phenotypes of the affected fetuses, although the inclusion criteria of our present review, 'at least two major anomalies from different anatomical systems' seems unlikely to be misconstrued.

There were also some limitations of the review processes used, given that the included studies were not reported series including only multisystem multiple anomalies, which made data extraction more complex. In addition, the methods for ES and its interpretation were not clearly specified in some series. In relation to tested individuals, ES can be applied using a solo or a trio approach, with only the proband or both parents together with the proband being sequenced, respectively. In this systematic review, 13 studies applied the trio approach, while solo samples were sequenced in two. To address this issue, our stratified meta-analysis found that the diagnostic yields were not statistically significantly different between these two approaches (trio: 35% *vs* solo: 31%).

In clinical practice, guidelines recommend trio analysis because it makes easier the interpretation of results and aids assignment of pathogenicity for detected sequence variants through exclusion of familial, most likely benign, genomic variants[37]. Recent improvements in neonatal ES have reduced the turnaround time to 14 days for rapid clinical ES[27]. However, there are as yet no recommendations regarding which part of the exome should be analyzed. Interpretation of the whole exome is cumbersome and far more complex than is the sequencing itself. For this reason, often, in clinical ES, the whole exome is sequenced but only the OMIM genes are interpreted. The contribution of whole-ES in the research setting is based on the fact that it enables identification of novel candidate genes critical to human development, and prediction of deleterious effects of variants in novel candidate genes that may not have been implicated previously in human disease, or for which the published data regarding an association with human disease may not yet be definitive.

This systematic review and meta-analysis has shown that ES applied in fetuses with multisystem structural anomalies enables identification of the causative gene in a third of cases. A policy of offering ES in such cases after normal CMA results seems reasonable. No differences were observed between the solo and trio approaches to ES. Further studies are needed to explore the role of genome sequencing in fetuses with these anomalies in order to further increase the diagnostic yield of next-generation sequencing.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figure S1 Funnel plot analysis for the asymmetry of publication among small studies.

Figure S2 Forest plots of the diagnostic yield after stratification by trio or solo approach.

Table S1 Affected anatomical systems observed in fetuses included in the 12 series reporting individual phenotypes ($n = 383$) and in their cases that were positive on exome sequencing ($n = 136$)

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Rendimiento diagnóstico de la secuenciación del exoma en fetos con malformaciones multisistémicas: revisión sistemática y metaanálisis

RESUMEN

Objetivo. Determinar el rendimiento diagnóstico de la secuenciación del exoma (SE) por encima del del análisis de microarrays cromosómicos (AMC) o del cariotipado en fetos con anomalías estructurales multisistémicas (al menos dos anomalías importantes en diferentes sistemas anatómicos).

Método. Se trata de una revisión sistemática realizada de acuerdo con las directrices PRISMA. Se hizo una búsqueda en PubMed, Web of Knowledge y en la base de datos Cochrane para identificar estudios que describían la SE, la secuenciación del genoma completo y/o de próxima generación en fetos con malformaciones multisistémicas. Se incluyeron estudios observacionales con cinco o más fetos elegibles. Un feto se consideró elegible para su inclusión si tenía al menos dos anomalías importantes de diferentes sistemas anatómicos y un resultado negativo del AMC o del cariotipado. Sólo se consideraron las variantes positivas clasificadas como probablemente patógenas o patógenas determinadas como causantes del fenotipo fetal. Un resultado negativo del AMC o del cariotipado fue tratado como el estándar de referencia. El rendimiento del diagnóstico del resultado primario se calculó mediante un análisis de proporción única utilizando un modelo de efectos aleatorios. Se realizó un análisis de subgrupos para comparar el rendimiento del diagnóstico del enfoque en solitario (solo feto secuenciado) con el del enfoque en trío (feto y ambos padres secuenciados).

Resultados. Se identificaron 17 artículos con datos sobre el rendimiento diagnóstico de la SE, incluidos 694 individuos con malformaciones multisistémicas. En general, se encontró una variante patogénica o probablemente patogénica potencialmente causante del fenotipo fetal en 213 fetos, lo que supone un rendimiento incremental de la SE del 33% (IC 95%, 27–40%). Un análisis estratificado mostró rendimientos del diagnóstico similares de la SE utilizando el enfoque en solitario (30%; IC 95%, 11–52%) y el enfoque en trío (35%; IC 95%, 26–44%).

Conclusiones. La SE aplicada a fetos con anomalías estructurales multisistémicas fue capaz de identificar un gen potencialmente causante cuando el AMC o el cariotipado no lo habían conseguido en un tercio adicional de los casos. No se observaron diferencias entre los enfoques en solitario y en trío para la SE.

外显子组测序在多系统畸形胎儿中的诊断率：系统评价和荟萃分析

摘要

目的在多系统结构性畸形的胎儿中（至少有不同解剖系统上的两大畸形），确定外显子组测序（ES）的诊断率超越于染色体微阵列分析（CMA）或染色体核型分析的诊断率之处。

方法这是按照 PRISMA 指导方针进行的一项系统评价。通过搜索 PubMed、知识网（Web of Knowledge）和实证医学资料库（Cochrane database），我们识别了描述 ES 全基因组和/或新一代测序技术针对多系统畸形胎儿的研究。这里包括涉及五个或五个以上符合条件的胎儿的观察性研究。包含在研究内符合条件的胎儿至少有不同解剖系统上的两大畸形和一个 CMA 或染色体核型分析的否定结果。只有分类为很可能的致病或发病的阳性变异型被确定为造成胎儿表型原因的才会被考虑。一个 CMA 或染色体核型分析的否定结果被当作参考标准。主要结局的诊断率通过单一比例分析进行计算（使用随机效应模型）。执行了一个亚组分析来比较单人方案（胎儿单独测序）的诊断率和三人方案（对胎儿和父母都测序）的诊断率。

结果 确定了 17 篇含有 ES 诊断率数据的文章，包含 694 名多系统畸形的个体。总的说来，一种可能造成胎儿表型的致病或很可能致病的变异型在 213 名胎儿身上找到，带来 ES33%的增量产出率（95% CI, 27 – 40%）。分层分析表明使用单人方案（30%; 95%CI, 11 – 52%）和三人方案（35%; 95%CI, 26 – 44%）的 ES 诊断率类似。

结论 应用于多系统结构性畸形胎儿的 ES，当 CMA 或染色体核型分析在额外三分之一的病例中失败时，能够识别一种可能的致病基因。对于 ES，在单人和三人方案之间没有观察到任何不同之处。