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Incremental yield of whole-genome sequencing over chromosomal microarray analysis and exome sequencing for congenital anomalies in prenatal period and infancy: systematic review and meta-analysis

N. SHREEVE[1#], C. SPROULE[2#], K. W. CHOY[3], Z. DONG[3], K. GAJEWSKA-KNAPIK[4], M. D. KILBY[5,6,7†] and F. MONE[8†]

[1]*Department of Obstetrics & Gynaecology, University of Cambridge, Cambridge, UK;* [2]*Department of Obstetrics & Gynaecology, South Eastern Health and Social Care Trust, Belfast, UK;* [3]*Department of Obstetrics & Gynaecology, The Chinese University of Hong Kong, Hong Kong, China;* [4]*Department of Obstetrics & Gynaecology, Cambridge University Hospitals, Cambridge, UK;* [5]*Fetal Medicine Centre, Birmingham Women's and Children's Foundation Trust, Birmingham, UK;* [6]*College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK;* [7]*Medical Genomics Research Group, Illumina, Cambridge, UK;* [8]*Centre for Public Health, Queen's University Belfast, Belfast, UK*

KEYWORDS: chromosomal microarray analysis; exome sequencing; fetal anomaly; neonatal; next-generation sequencing; prenatal; whole-genome sequencing

CONTRIBUTION

What are the novel findings of this work?
There is presently no evidence of a significant increase in incremental yield with whole-genome sequencing (WGS) over the sequential approach of quantitative fluorescence polymerase chain reaction (QF-PCR)/chromosomal microarray analysis (CMA) and exome sequencing (ES) when investigating congenital malformations in the perinatal period or during infancy. However, WGS requires less DNA and has a potentially shorter turnaround time compared with sequential QF-PCR/CMA and ES.

What are the clinical implications of this work?
Presently, there is inadequate evidence to advocate WGS over ES in the investigation of congenital malformations, either prenatally, neonatally or in infancy.

ABSTRACT

Objectives *First, to determine the incremental yield of whole-genome sequencing (WGS) over quantitative fluorescence polymerase chain reaction (QF-PCR)/chromosomal microarray analysis (CMA) with and without exome sequencing (ES) in fetuses, neonates and infants with a congenital anomaly that was or could have been detected on prenatal ultrasound. Second, to evaluate the turnaround time (TAT) and quantity of DNA required for testing using these pathways.*

Methods *This review was registered prospectively in December 2022. Ovid MEDLINE, EMBASE, MEDLINE (Web of Science), The Cochrane Library and ClinicalTrials.gov databases were searched electronically (January 2010 to December 2022). Inclusion criteria were cohort studies including three or more fetuses, neonates or infants with (i) one or more congenital anomalies; (ii) an anomaly which was or would have been detectable on prenatal ultrasound; and (iii) negative QF-PCR and CMA. In instances in which the CMA result was unavailable, all cases of causative pathogenic copy number variants > 50 kb were excluded, as these would have been detectable on standard prenatal CMA. Pooled incremental yield was determined using a random-effects model and heterogeneity was assessed using Higgins' I^2 test. Subanalyses were performed based on pre- or postnatal cohorts, cases with multisystem anomalies and those meeting the NHS England prenatal ES inclusion criteria.*

Results *A total of 18 studies incorporating 902 eligible cases were included, of which eight (44.4%) studies focused on prenatal cohorts, incorporating 755 cases, and the remaining studies focused on fetuses*

Correspondence to: Dr F. Mone, Centre for Public Health, Queen's University Belfast, Institute of Clinical Science Block A, Royal Victoria Hospital, Grosvenor Rd, Belfast BT12 6BA, UK (e-mail: f.mone@qub.ac.uk)
#N.S. and C.S. are joint first authors. †M.D.K. and F.M. are joint senior authors.

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undergoing postmortem testing or neonates/infants with congenital structural anomalies, constituting the postnatal cohort. The incremental yield of WGS over QF-PCR/CMA was 26% (95% CI, 18–36%) ($I^2 = 86\%$), 16% (95% CI, 9–24%) ($I^2 = 85\%$) and 39% (95% CI, 27–51%) ($I^2 = 53\%$) for all, prenatal and postnatal cases, respectively. The incremental yield increased in cases in which sequencing was performed in line with the NHS England prenatal ES criteria (32% (95% CI, 22–42%); $I^2 = 70\%$) and in those with multisystem anomalies (30% (95% CI, 19–43%); $I^2 = 65\%$). The incremental yield of WGS for variants of uncertain significance (VUS) was 18% (95% CI, 7–33%) ($I^2 = 74\%$). The incremental yield of WGS over QF-PCR/CMA and ES was 1% (95% CI, 0–4%) ($I^2 = 47\%$). The pooled median TAT of WGS was 18 (range, 1–912) days, and the quantity of DNA required was 100 ± 0 ng for WGS and 350 ± 50 ng for QF-PCR/CMA and ES (P = 0.03).

Conclusion *While WGS in cases with congenital anomaly holds great promise, its incremental yield over ES is yet to be demonstrated. However, the laboratory pathway for WGS requires less DNA with a potentially faster TAT compared with sequential QF-PCR/CMA and ES. There was a relatively high rate of VUS using WGS. © 2023 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Next-generation sequencing is a rapidly expanding area in the prenatal and postnatal investigation of congenital anomalies[1]. This relatively novel approach to prenatal diagnosis has become available only within the last 5 years. The bulk of evidence obtained regarding clinical utility applies to exome sequencing (ES), targeting the protein-coding regions of the genome, which account for only 1–2% of the total human genome but contain around 85% of disease-causing variants[2,3]. The diagnostic yield of ES has been described as dependent on the type of fetal anomaly and whether multiple abnormalities are identified[4,5]. ES has been reported to provide an incremental yield of 31% over quantitative fluorescence polymerase chain reaction (QF-PCR) and chromosomal microarray analysis (CMA) for any congenital anomaly, with the greatest yield demonstrated for suspected skeletal dysplasia (53%) and neuromuscular disorders (37%)[4–8].

Whole-genome sequencing (WGS) allows assessment of the entire genome and theoretically should offer additional diagnostic capability due to its ability to capture information such as pathogenic copy number variants (CNV) with greater resolution compared with that of CMA, as well as intronic variants, repeat expansions, structural DNA alterations and mitochondrial disorders[9–11]. Additionally, it is potentially less affected by GC-rich regions and offers more uniform coverage, requiring an overall lower quantity of DNA compared with the sequential QF-PCR/CMA and ES approach[12–15]. However, despite its increasingly competitive costs, there remain concerns related to the cost-effectiveness of WGS and postsequencing interpretation of findings[16,17]. Evidence demonstrating the increased clinical utility of WGS in the diagnosis of rare genetic disease originates primarily from pediatric cohort studies, while data on prenatal diagnosis are derived from research studies and generally demonstrate a modest incremental yield over ES[18–20]. To date, it has not been established whether there is advantage in performing WGS from the outset instead of sequential QF-PCR/CMA and ES; therefore, in the absence of such evidence, the latter remains the recommended pathway, with CMA typically run in parallel with ES.

The role of WGS in a prenatal setting is promising, but has yet to be elucidated fully[21]. The primary objective of this systematic review and meta-analysis was to determine the incremental yield of WGS over QF-PCR/CMA with or without ES in the investigation of fetuses, neonates and infants (up to 1 year of age) with congenital anomalies (that were or would have been detectable prenatally). The secondary objective was to evaluate the turnaround time (TAT) and quantity of DNA required for testing using WGS *vs* QF-PCR/CMA and ES.

METHODS

Information sources

This systematic review was performed in a standardized fashion in line with the recommended methods for systematic reviews and international PRISMA guidance and was registered prospectively on 6 December 2022 (PROSPERO No. CRD42022380483)[22,23]. Ovid MEDLINE, EMBASE, MEDLINE (Web of Science), The Cochrane Library and ClinicalTrials.gov were searched electronically for relevant citations from January 2010 (WGS was not widely utilized prior to this) until December 2022. The search strategy consisted of the following relevant medical subject headings (MeSH) terms, keywords and word variants: ('next generation sequencing', 'sequence analysis', 'DNA', 'high-throughput nucleotide sequencing', 'exome' OR 'genome') AND ('congenital anomaly', 'defect', 'malformation' OR 'abnormality') AND ('fetus', 'child', 'infant', 'prenatal' OR 'fetal'). The search within ClinicalTrials.gov consisted of ('congenital anomaly') AND ('whole-genome sequencing') AND ('completed'). Reference lists of relevant articles were searched manually and experts in prenatal genomics were also contacted to identify further relevant studies. The full search strategy is available from the corresponding author upon request.

Study selection

Inclusion criteria were prospective or retrospective cohort studies including three or more cases of fetuses, neonates or infants with: (i) one or more congenital anomaly (including nuchal translucency > 3.5 mm and fetal growth

restriction); (ii) congenital anomalies that would have been detectable on prenatal ultrasound; and (iii) negative QF-PCR and CMA. In instances in which the CMA result was unavailable, all cases of causative pathogenic CNV > 50 kb were excluded, as these would have been detectable on standard prenatal CMA. In relation to the postnatal cohort, two fetal medicine specialists (F.M. and M.D.K.) reached a consensus on which cases would have been detectable using standard fetal ultrasound or magnetic resonance imaging. For example, seizures or a bilobed right lung would not have been included as findings detectable prenatally.

All study abstracts were screened independently by two reviewers (N.S. and C.S.) using Covidence[24], a web-based collaboration software platform. Any disputes were discussed with the senior authors (M.D.K. and F.M.), and a resolution was reached. Full-text analysis was then performed on selected studies, and authors were contacted if further information was required prior to inclusion for data extraction.

Data extraction and quality assessment

Two reviewers (N.S. and F.M.) extracted independently data on study characteristics and outcome using a common proforma. Quality assessment was performed using modified Standards for Reporting of Diagnostic Accuracy (STARD) criteria[25]. Where available, the following data were extracted from studies: clinical characteristics, ultrasound phenotype, sequencing approach, reported sequencing variants, amount of fetal DNA required and TAT. Subgroup analyses were planned on cases with multisystem abnormalities and those with anomalies selected in line with the NHS England R21 prenatal ES criteria, which is a robust evidence-based approach to selecting fetal anomaly cases with optimal diagnostic yield for prenatal ES. Inclusion criteria for this pathway are as follows: (i) fetuses with multiple anomalies; (ii) suspected skeletal dysplasia (including isolated short long bones < 3rd percentile); (iii) large echogenic kidneys with a normal bladder; (iv) major central nervous system abnormalities (excluding neural tube defects); (v) multiple contractures (excluding isolated bilateral talipes); (vi) nuchal translucency > 6.5 mm plus another anomaly (including a minor finding) with normal CMA findings; (vii) isolated immune hydrops fetalis, defined as fluid/edema in at least two compartments with normal microarray analysis; (viii) small-for-gestational-age fetuses in which all measurements are < 3rd percentile with confirmed early ultrasound-estimated due date and no evidence of placental insufficiency. These criteria were used, as to our knowledge these are the only known existing ES selection criteria available at present[26].

Statistical analysis

Descriptive tables were produced, detailing study design, cohort characteristics and next-generation sequencing approaches. Statistical analysis was performed by a biostatistician using STATA version 18 (Stata Corp., College Station, TX, USA). Meta-analysis of single proportions was performed to demonstrate the incremental yield using Freeman–Tukey double arcsine transformation, which was checked using a second method (a logistic-normal random-effects model) with similar results. This approach has been described previously[27]. A random-effects model was used for pooling the effect sizes, and their 95% CI was calculated. Results were displayed in forest plots with the corresponding 95% CI. Publication bias was assessed graphically using funnel plots to demonstrate the relationship between the study effect size and precision. Heterogeneity was assessed graphically within the forest plots and statistically using Higgins' I^2 test.

RESULTS

Systematic review and meta-analysis

In total, 18 studies incorporating 1284 cases (probands) met the inclusion criteria (Figure 1)[11,28–44]. Of the 1284 cases, subselection based on cases in which the congenital anomaly was identifiable prenatally led to a final number

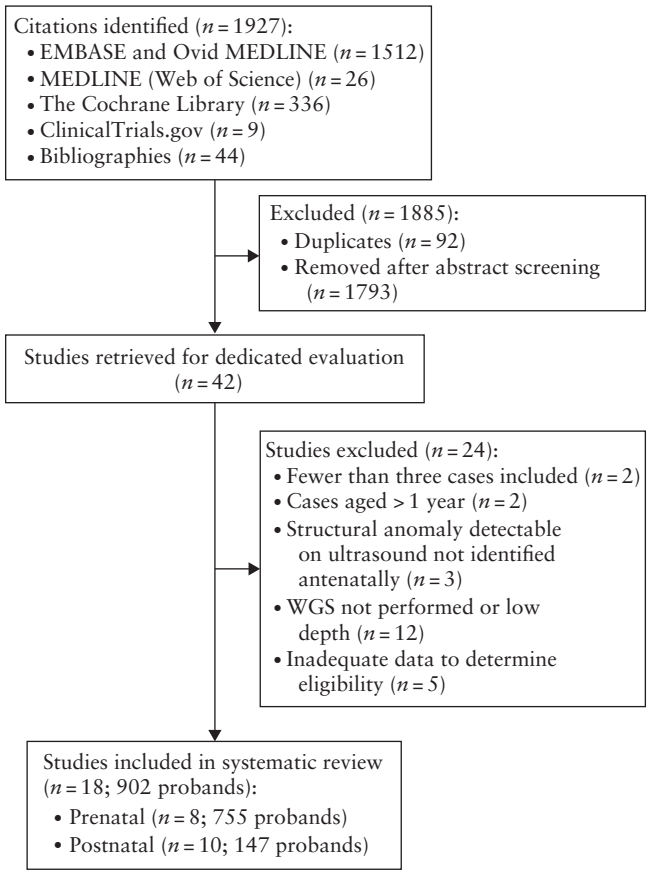

Figure 1 Flowchart summarizing inclusion in systematic review of studies on incremental yield of whole-genome sequencing (WGS) over quantitative fluorescence polymerase chain reaction/chromosomal microarray analysis with or without exome sequencing in fetuses or neonates/infants with prenatally detectable structural anomalies.

of 902 (70.2%) included in the final analysis. Table 1 displays study characteristics and Figure 2 shows the overall quality assessment of the studies. Of the 18 studies, 11 (61.1%) performed WGS on a research basis, four (22.2%) did it on a clinical basis, while, for the remaining studies, it was uncertain or not specified. When phenotypic information on the fetus or infant was incomplete (12 studies), corresponding authors were contacted to request further data; of these, six (50%) authors responded and one provided further data for inclusion in the analysis[30]. Eight (44.4%) studies reported on 755 fetuses with an anomaly (prenatal cohort) and 10 (55.6%) studies included 15 anomalous fetuses undergoing postmortem testing ($n = 1$) and 132

Table 1 Characteristics of studies included in systematic review

Study	*Study design*	*Study cohort*	*WGS approach**	*Included/total cases* (n)
Armes (2018)[28]	Retro	Prenatal death cases with US anomalies (mixed phenotype) undergoing autopsy	80×, trio, patterned nanoarrays of self-assembling DNA nanoballs	15/16
Cao (2022)[29]	Prosp	Selected fetuses with congenital heart disease ± other US anomalies and no diagnosis on standard genetic testing	30×, trio, MGISEQ-2000 (MGI)	13/13
Choy (2019)[30]	Retro	Fetuses with increased NT (≥ 3.5 mm) ± other anomalies on US undergoing routine prenatal diagnosis	30×, singleton, MGISEQ-2000 (MGI)	42/50
Denommé-Pichon (2022)[31]	Prosp	Critically ill infants/newborns with structural anomaly detectable prenatally†	Coverage NS, trio, NovaSeq 6000 (Illumina)	16/37
Farnaes (2018)[32]	Retro	Critically ill infants with structural anomaly detectable prenatally and negative standard testing	45×, trio, HiSeq X (Illumina)	19/42
French (2019)[33]	Prosp	Critically ill neonates in NICU with structural anomaly detectable prenatally and negative standard testing	30–40×, trio, Illumina NS	22/195
Hu (2023)[34]	Prosp	Fetuses with mixed phenotype subjected to WGS and CMA in parallel	30×, singleton and trio, DNBSEQ-T7 (MGI)	165/185
Lowther (2022)[11]	Retro	Fetuses with structural anomaly (mixed phenotype) prescreened with one or more standard diagnostic test	> 30×, trio, Illumina sequencing, Broad Institute Genomics Platform	249/249
Lumaka (2023)[35]	Retro	Infants < 1 year with structural anomalies (mixed phenotype) and negative standard testing	Coverage NS, trio, NovaSeq 6000 (Illumina)	6/21
Mestek-Boukhibar (2018)[36]	Prosp	Critically ill selected infants with congenital anomalies detectable prenatally†	Coverage NS, trio, TruSeq PCR-free prep (Illumina), HiSeq 2500 (Illumina)	13/24
Petrikin (2018)[37]	Prosp	Critically ill selected infants < 4 months with congenital anomalies detectable prenatally and negative CMA	40×, trio, HiSeq 2500/4000 (Illumina)	11/32
So (2022)[38]	Retro	Selected infants < 1 year with congenital anomalies detectable prenatally†	30×, trio, NovaSeq (Illumina), HiSeq X Ten (Illumina) or MGISEQ-2000 (MGI)	13/29
van Diemen (2017)[39]	Prosp	Critically ill selected infants with congenital anomalies detectable prenatally†	36×, trio, gene panel, NEBNext DNA Library Prep, HiSeq 2500 or NextSeq500 (Illumina)	6/23
Wang (2022)[40]	Prosp	Fetuses with mixed phenotype and normal QF-PCR and CMA	> 30×, singleton, HiSeq X (Illumina)	34/37
Westenius (2022)[41]	Retro	Selected fetuses with prenatally diagnosed non-immune hydrops fetalis, negative for trisomies and CNV > 50 kb, referred for clinical WGS	Coverage NS, trio, Hiseq X Ten ($n = 10$) or NovaSeq 6000 ($n = 13$) (Illumina)	22/23
Willig (2015)[42]	Retro	Critically ill selected infants < 4 months with congenital anomalies detectable prenatally†	40×, trio, TruSeq PCR Free prep (Illumina), HiSeq 2500 (Illumina)	26/35
Yang (2022)[43]	Prosp	Fetuses with CNS anomalies†	41.9×, BGISEQ-500 (BGI) or MGISEQ-2000 (MGI)	127/162
Zhou (2021)[44]	Prosp	Fetuses with structural anomalies and FGR (mixed phenotype) and negative CMA, undergoing ES and WGS	40×, trio, MGISEQ-2000 (MGI)	103/111

Only first author is given for each study. *Whole-genome sequencing (WGS) approach outlined as described by original study. †Excluding those caused by aneuploidy or pathogenic copy number variant >50 kb. BGI, BGI Genomics, Shenzhen, China; CMA, chromosomal microarray analysis; CNV, copy number variant; ES, exome sequencing; FGR, fetal growth restriction; Illumina, Illumina, San Diego, CA, USA; MGI, MGI Tech. Co. Ltd, Shenzhen, China; NICU, neonatal intensive care unit; NS, not specified; NT, nuchal translucency; PCR, polymerase chain reaction; Prosp, prospective; QF-PCR, quantitative fluorescence polymerase chain reaction; Retro, retrospective; US, ultrasound.

critically ill neonates/infants (from birth to 1 year of age) with testing based on clinical findings ($n = 9$) (postnatal cohort). Table S1 outlines the pathogenic and likely pathogenic causative variants identified.

WGS *vs* QF-PCR/CMA

Overall cohort

Table 2 and Figures 3, S1 and S2 demonstrate the incremental yield of WGS over QF-PCR/CMA for all subgroup analyses in combined, prenatal and postnatal cohorts, and Figure S3 shows the corresponding funnel plots. The overall incremental yield of WGS over QF-PCR/CMA across the combined prenatal and postnatal cohorts was 26% (95% CI, 18–36%) ($I^2 = 86\%$). The incremental yield increased in cases with multisystem anomalies and

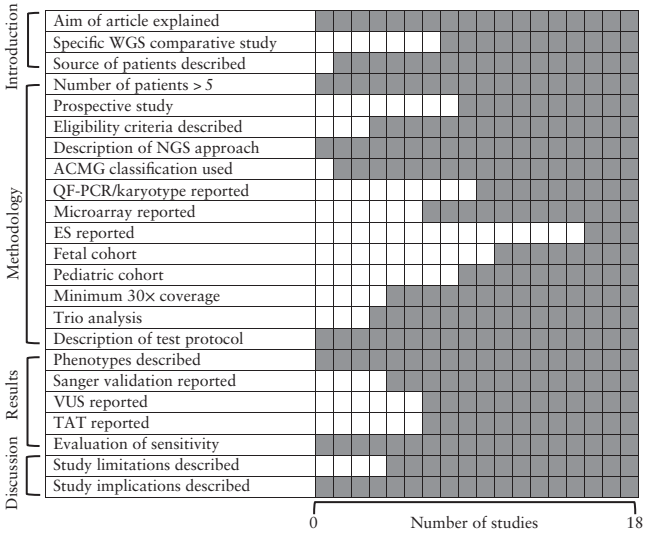

Figure 2 Quality assessment of studies included in systematic review using modified Standards for Reporting of Diagnostic Accuracy criteria. □, no; ■, yes. ACMG, American College of Medical Genetics and Genomics; ES, exome sequencing; NGS, next-generation sequencing; QF-PCR, quantitative fluorescence polymerase chain reaction; TAT, turnaround time; VUS, variant of uncertain significance; WGS, whole-genome sequencing.

Table 2 Incremental yield of whole-genome sequencing compared with quantitative fluorescence polymerase chain reaction/chromosomal microarray analysis with resolution of 50 kb

Anomaly group	*Incremental yield (%)*	*Heterogeneity (%)*
All		
All cases	26 (18–36)	86
Prenatal	16 (9–24)	85
Postnatal	39 (27–51)	53
Multisystem		
All cases	30 (19–43)	65
Prenatal	23 (12–36)	70
Postnatal	43 (27–59)	21
R21 selected		
All cases	32 (22–42)	70
Prenatal	22 (14–31)	65
Postnatal	48 (37–60)	0

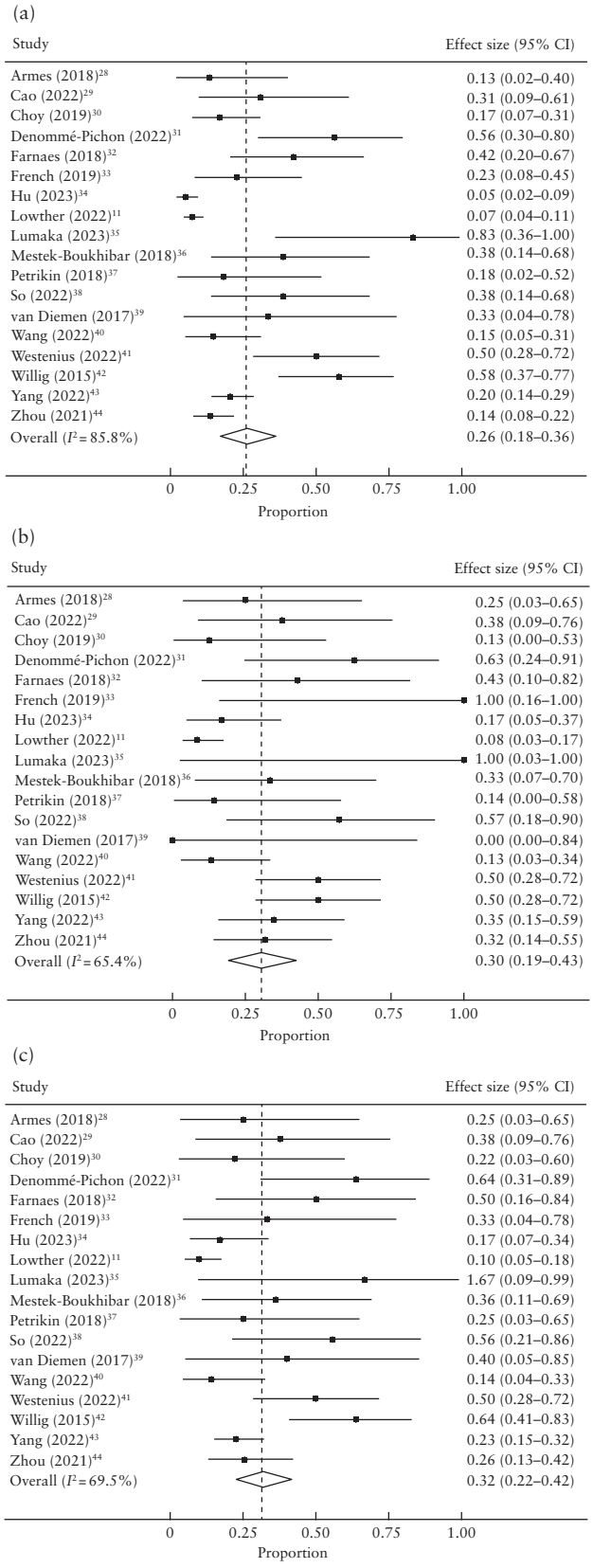

Figure 3 Forest plots showing incremental yield of whole-genome sequencing over quantitative fluorescence polymerase chain reaction/chromosomal microarray analysis in prenatal and postnatal cohorts, in all cases (a), those with multisystem structural anomalies (b) and those with anomalies categorized according to NHS England R21 prenatal exome sequencing criteria (c). Only first author is given for each study.

anomalies categorized by NHS England R21 prenatal ES criteria. When considering anatomical systems, abnormalities of the musculoskeletal system (36% (95% CI, 20–52%); $I^2 = 67\%$) and central nervous system (30% (95% CI, 17–42%); $I^2 = 65\%$) had the greatest incremental yield (Table S2). The pooled incremental yield for variants of uncertain significance (VUS) was 18% (95% CI, 7–33%) ($I^2 = 74\%$) (Figure S4). The most commonly identified genetic syndrome was Noonan syndrome (9.9% (15/151)), and pathogenic variants occurred predominantly *de novo* (61.2% (71/116)) and in monoallelic (autosomal dominant) inheritance genes (77.5% (117/151)).

Prenatal cohort

When focusing on the prenatal cohort only, the incremental yield of WGS over QF-PCR/CMA was 16% (95% CI, 9–24%) ($I^2 = 85\%$). In fetuses in which multiple systems were affected, this increased to 23% (95% CI, 12–36%) ($I^2 = 70\%$). In fetuses meeting the criteria for NHS England R21 prenatal ES, the incremental yield of WGS was 22% (95% CI, 14–31%) ($I^2 = 65\%$). The most common genetic diagnostic syndrome was Noonan syndrome (11.8% (11/93)).

Postnatal cohort

In the postnatal cohort, the incremental yield of WGS over QF-PCR/CMA was 39% (95% CI, 27–51%) ($I^2 = 53\%$). In cases with multisystem anomalies, this increased to 43% (95% CI, 27–59%) ($I^2 = 21\%$). In cases meeting the criteria for NHS England R21 ES in the prenatal period, the incremental yield of WGS increased further to 48% (95% CI, 37–60%) ($I^2 = 0\%$). The most common genetic syndromes were Noonan syndrome, epileptic encephalopathy and CHARGE syndrome (each affecting 6.9% (4/58) of cases).

WGS *vs* QF-PCR/CMA and ES

Three studies were included in the meta-analysis comparing the incremental yield of WGS over QF-PCR/CMA and ES, both pre- and postnatally. The incremental yield of WGS over QF-PCR/CMA and ES was only 1% (95% CI, 0–4%) ($I^2 = 47\%$) (Figure 4). The pooled median TAT for WGS was 18 (range, 1–912) days, with only one study reporting on the TAT for QF-PCR/CMA and ES, which was 31 ± 8 days, meaning statistical comparison between groups was not feasible. The pooled mean quantity of DNA required for WGS *vs* QF-PCR/CMA and ES from all three studies was 100 ± 0 ng *vs* 350 ± 50 ng, respectively ($P = 0.03$).

DISCUSSION

This meta-analysis demonstrates a significant incremental yield with WGS over QF-PCR/CMA in the genomic investigation of congenital anomaly. This was greatest

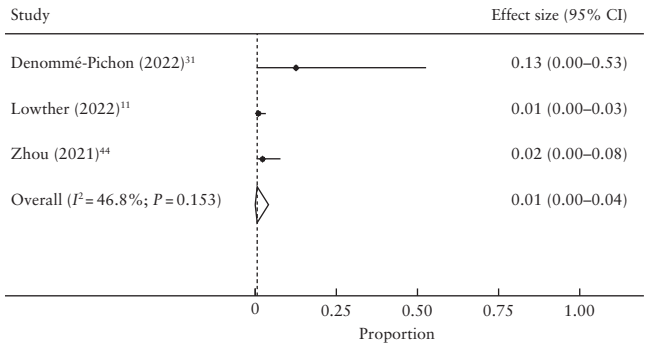

Figure 4 Forest plot showing incremental yield of whole-genome sequencing over quantitative fluorescence polymerase chain reaction/chromosomal microarray analysis and exome sequencing in all cases of fetal anomaly. Only first author is given for each study.

in the postnatal cohort and when limited to cases with multisystem anomalies and those selected in line with NHS England R21 prenatal ES inclusion criteria[26]. Presently, there is insufficient evidence to advocate the use of WGS over QF-PCR/CMA and ES (either prenatally or postnatally). However, WGS appears to require less DNA than a stepwise testing strategy (QF-PCR/CMA and ES) and potentially provides results in a shorter timeframe to facilitate decision-making.

These findings are comparable with those reported for prenatal ES with a pooled incremental yield over CMA of 31% (95% CI, 26–36%) and a relatively high yield in cases with musculoskeletal and central nervous system anomalies[45–49]. It is also in keeping with previous comparisons of ES and WGS in unselected pediatric cohorts[50]. The yield of WGS in an unscreened population is likely to be higher compared with that demonstrated by this review, and inclusion of diagnoses secondary to aneuploidy, unbalanced structural variants, loss of heterozygosity and CNV > 100 kb, all of which are detectable on WGS, would likely increase the yield over ES[11]. This and the fact that WGS requires less DNA and potentially shorter TAT justify its laboratory use and potential cost-effectiveness over and above stepwise testing[15,51,52]. In addition, the wet laboratory process of WGS is more rapid and comprehensive than that of ES.

It is well established that WGS serves presently as an optimal testing strategy in the pediatric investigation of critically ill children, those with intellectual impairment/developmental delay and those with congenital anomalies[53–56]. This is demonstrated by the greater number of cases and higher incremental yield in the postnatal than in the prenatal cohort in this review. In pediatric cohorts, it is more likely that detailed phenotypic information and classification obtained will aid selection of probands, whereas, prenatally, selection is more difficult and affected by evolving fetal phenotypes. This assertion is in part supported by a recent study[57], which demonstrated a significantly higher yield in the postnatal group when comparing the prenatal *vs* postnatal ES diagnostic yield in subjects in which a genetic disorder was suspected. In this study, some variants could not be classified

as causative, due to the need for associated clinical or biochemical confirmation (e.g. Case SR728 with X-linked hypophosphatemic rickets and Case SR734 with early infantile epileptic encephalopathy Type 7 (Table S1)). Although they were included as pathogenic variants in this review, it is unlikely that they would have been judged so prospectively in the prenatal period due to the impact on bioinformatic interpretational criteria to assign pathogenicity[58]. The availability of these criteria will differ between prenatal and postnatal cohorts, making it more likely that such variant would be classified as VUS and potentially upgraded postnatally[59].

Prenatal cases should be handled with greater caution, as the implications of a pathogenic variant may alter the course of pregnancy and the extent of a phenotype may not be reflected fully by ultrasound findings. Indeed, prenatal inheritance filtering approaches can be challenging and phenotypes can be dynamic, with new information changing the variant classification[60]. Prenatally, autosomal aneuploidy is common and detected by QF-PCR with a TAT of 48 h. In many studies, QF-PCR is performed before undertaking WGS[44]. As it stands, significant bioinformatic challenges persist in genomic medicine. The increase in sequencing capacity has accelerated its diagnostic power, but there is often little or inconclusive supporting functional evidence for the ever-increasing number of novel suspected pathogenic variants[61]. Indeed, it is also likely that the incremental yield of WGS over QF-PCR/CMA is similar to that of ES, as current bioinformatic tools for interpretation of non-coding areas of the genome and the implications of complex structural variants are not advanced enough to draw causative genotype–phenotype correlations. In the future, the incorporation of well-evaluated secondary and tertiary analytical software, with or without the use of artificial intelligence, and automated genome interpretation models may significantly aid this process[62]. It also justifies the need for deep phenotyping with sequential scans and complementary imaging tools (fetal magnetic resonance imaging)[63]. The health economics of WGS needs consideration, as does selection of cases, and it is promising to see that those classified as per the NHS England R21 prenatal ES criteria had the greatest yield, supporting them as robust selection criteria[26].

Although not directly compared with ES, the pooled incremental yield for VUS using WGS in this study was greater when compared with that using ES in other systematic reviews[7,8]. This is likely due to the post-WGS interpretational challenges, which may affect pre- and post-test counseling[2,64]. It is also interesting to note that a recent study of over a million genomes has noted that, with increasing use, the VUS rate is lower when WGS is utilized over classic genomic panels[65].

As potentially transformative newborn WGS screening projects aiming to identify rare genetic conditions in neonates and children begin to emerge[66], this analysis is both clinically relevant and timely. To our knowledge, this is the first meta-analysis to compare WGS with ES and QF-PCR/CMA. The strength of this study lies in our attempts to optimize numbers through inclusion of postnatal cohorts and seeking complete datasets from authors. Nevertheless, the primary limitation of the study is the low number of cases included and that there was a high degree of heterogeneity despite adoption of a random-effects model and subanalyses. Such heterogeneity is likely due to inclusion of postnatal cohorts and has been seen in similar systematic reviews, but it also means that the results should be interpreted with caution[45]. A further limitation is the fact that most of the studies performed WGS on a research basis, hence TAT may have been more protracted than in a clinical setting. Further studies comparing WGS and ES prenatally are required to delineate whether WGS has any incremental yield. Studies in which WGS serves as the one encompassing test evaluating its feasibility and acceptability are also needed. Finally, a cost-effectiveness analysis comparing WGS *vs* QF-PCR/CMA and ES is required, and efforts should be made to optimize access to QF-PCR/CMA and ES in the first instance in resource-limited settings.

In conclusion, while WGS holds great promise, its utility in a prenatal setting over ES has not been elucidated fully. Compared with a stepwise approach of QF-PCR/CMA and ES, the laboratory-based pathway of WGS utilizes less fetal DNA and potentially has faster TAT, although it is associated with more VUS. As our interpretational knowledge of non-coding regions and complex structural variants advances, the diagnostic yield will likely increase and the VUS rate will fall. However, presently, there is inadequate evidence to endorse the application of WGS prenatally over and above ES.

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SUPPORTING INFORMATION ON THE INTERNET

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

Figures S1 and S2 Forest plots showing incremental yield of whole-genome sequencing over quantitative fluorescence polymerase chain reaction/chromosomal microarray analysis in prenatal (Figure S1) and postnatal (Figure S2) cohort, in all cases (a), cases with multisystem structural anomalies (b) and cases with anomalies categorized according to NHS England R21 prenatal exome sequencing criteria (c).

Figure S3 Funnel plots for all studies reporting on incremental yield of whole-genome sequencing over quantitative fluorescence polymerase chain reaction/chromosomal microarray analysis in prenatal and postnatal cohorts in all cases (a), those with multisystem anomalies (b) and those with anomalies categorized according to NHS England R21 prenatal exome sequencing criteria (c).

Figure S4 Forest plot showing pooled incremental yield of whole-genome sequencing over quantitative fluorescence polymerase chain reaction/chromosomal microarray analysis for variants of uncertain significance in all cases in prenatal and postnatal cohorts.

Table S1 All reported causative pathogenic and likely pathogenic variants

Table S2 Incremental yield of whole-genome sequencing over quantitative fluorescence polymerase chain reaction/chromosomal microarray analysis, according to anatomical system

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Rendimiento incremental de la secuenciación del genoma completo sobre el análisis de microarrays cromosómicos y la secuenciación del exoma para anomalías congénitas en el periodo prenatal y la infancia: revisión sistemática y metaanálisis

RESUMEN

Objetivos. En primer lugar, determinar el rendimiento incremental de la secuenciación del genoma completo (SGC) con respecto a la reacción en cadena de la polimerasa con fluorescencia cuantitativa (QF-PCR, por sus siglas en inglés)/análisis de microarrays cromosómicos (AMC) con y sin secuenciación del exoma (SE) en fetos, neonatos y lactantes con una anomalía congénita que fue detectada en la ecografía prenatal o que podría haberlo sido. En segundo lugar, evaluar el tiempo de respuesta (TAT, por sus siglas en inglés) y la cantidad de ADN necesario para realizar las pruebas utilizando estas opciones.

Métodos. Esta revisión se registró de forma prospectiva en diciembre de 2022. Se realizaron búsquedas electrónicas en las bases de datos Ovid MEDLINE, EMBASE, MEDLINE (Web of Science), The Cochrane Library y ClinicalTrials.gov (desde enero de 2010 hasta diciembre de 2022). Los criterios de inclusión fueron estudios de cohortes que incluyeran tres o más fetos, neonatos o lactantes con (i) una o más anomalías congénitas; (ii) una anomalía que se pudo detectar en la ecografía prenatal, o que hubiera podido serlo; y (iii) pruebas QF-PCR y de AMC negativas. En los casos en los que no se disponía del resultado del AMC, se excluyeron todos los casos de variantes patógenas causales del número de copias >50 kb, ya que habrían sido detectables en el AMC prenatal estándar. El rendimiento incremental combinado se determinó mediante un modelo de efectos aleatorios y la heterogeneidad se evaluó mediante la prueba I2 de Higgins. Se realizaron subanálisis basados en cohortes pre- o posnatales, casos con anomalías multisistémicas y aquellos que cumplían los criterios de inclusión de la SE prenatal del Servicio Nacional de Salud (NHS, por sus siglas en inglés) de Inglaterra.

Resultados. Se incluyeron un total de 18 estudios que incluían 902 casos elegibles, de los cuales ocho estudios (44,4%) se centraban en cohortes prenatales, que incluían 755 casos, y los estudios restantes se centraban en fetos sometidos a pruebas póstumas o neonatos/lactantes con anomalías estructurales congénitas, constituyendo la cohorte posnatal. El rendimiento incremental de la SGC sobre la QF-PCR/AMC fue del 26% (IC 95%, 18–36%) (I2 =86%), 16% (IC 95%, 9–24%) (I2 =85%) y 39% (IC 95%, 27–51%) (I2 =53%) para todos los casos, prenatales y posnatales, respectivamente. El rendimiento incremental aumentó en los casos en los que la secuenciación se realizó de acuerdo con los criterios de SE prenatal del NHS de Inglaterra (32% [IC 95%, 22–42%]); I2 =70%) y en aquellos con anomalías multisistémicas (30% (IC 95%, 19–43%); I2 =65%). El rendimiento incremental de la SGC para variantes de significación incierta (VSI) fue del 18% (IC 95%, 7–33%) (I2 =74%). El rendimiento incremental de la SGC sobre el QF-PCR/AMC y la SE fue del 1% (IC 95%, 0–4%) (I2 =47%). La mediana del TAT combinada de la SGC fue de 18 (rango, 1–912) días, y la cantidad de ADN requerida fue de 100}0 ng para SGC y 350}50 ng para la QF-PCR/AMC y la SE (P=0,03).

Conclusión. Aunque la SGC en casos con anomalías congénitas es muy prometedora, su rendimiento incremental sobre la SE está aún por demostrar. Sin embargo, la prueba de laboratorio para la SGC requiere menos ADN con un TAT potencialmente más rápido en comparación con la QF-PCR/AMC y la SE secuenciales. Se observó una tasa relativamente elevada de VSI mediante la SGC.

产前和婴儿期先天性异常的全基因组测序相比染色体微阵列分析和外显子组测序的增量收益：系统综述和荟萃分析

摘要

目的 首先，确定在产前超声已检测或可检测到先天性异常的胎儿、新生儿和婴儿中，全基因组测序（WGS）相比定量荧光聚合酶链反应（QF-PCR）/染色体微阵列分析（CMA）（含或不含外显子组测序（ES））的增量收益。其次，评价使用这些方法进行检测所需的出报告时间（TAT）和 DNA 量。

方法 本综述于 2022 年 12 月进行了前瞻性注册。对 Ovid MEDLINE、EMBASE、MEDLINE（Web of Science）、The Cochrane Library 和 ClinicalTrials.gov 数据库进行了电子检索（2010 年 1 月至 2022 年 12 月）。纳入标准为包括三例或更多具有以下情况的胎儿、新生儿或婴儿的队列研究：(i) 存在一项或多项先天性异常；(ii) 存在一项通过产前超声检查发现或可通过超声检查发现的异常；(iii) QF-PCR 和 CMA 结果为阴性。在无法获得 CMA 结果的情况下，所有致病性拷贝数变异大于 50 kb 的病例均被排除，因为标准的产前 CMA 将会检测出这些变异。使用随机效应模型确定汇总增量收益，并使用 Higgins' I^2 量表评估异质性。根据产前或产后队列、多系统异常病例以及符合英国国家医疗服务系统产前 ES 纳入标准的病例进行了子分析。

结果 共纳入了 18 项研究，含 902 个符合条件的病例，其中 8 项研究（44.4%）侧重于产前队列，含 755 个病例，其余研究侧重于接受死后检测的胎儿或患有先天性结构异常的新生儿/婴儿，构成产后队列。在所有病例、产前病例和产后病例中，WGS 相比 QF-PCR/CMA 的增量收益分别为 26%（95% CI，18–36%）（I2 =86%）、16%（95% CI，9–24%）（I2 =85%）和 39%（95% CI，27–51%）（I2 =53%）。按照英国国家医疗服务系统产前 ES 标准进行测序的病例（32%（95% CI，22–42%）；I2 =70%）和多系统异常病例（30%（95% CI，19–43%）；I2 =65%）的增量收益有所增加。WGS 对意义不确定的变异（VUS）的增量收益为 18%（95% CI，7–33%）（I2 =74%）。与 QF-PCR/CMA 和 ES 相比，WGS 的增量收益为 1%（95% CI，0–4%）（I2 =47%）。WGS 的集合中位 TAT 为 18 天（范围：1–912 天），WGS 所需的 DNA 量为 100}0 ng，QF-PCR/CMA 和 ES 则为 350}50 ng（P=0.03）。

结论 虽然 WGS 在先天性异常病例中大有可为，但其相比 ES 的增量收益仍有待证实。然而，与连续 QF-PCR/CMA 和 ES 相比，WGS 的实验室检测方法需要的 DNA 量更少，而且 TAT 可能更快。使用 WGS 的 VUS 率相对较高。