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Promises, pitfalls and practicalities of prenatal whole exome sequencing

Sunayna Best¹, Karen Wou², Neeta Vora³, Ignatia B. Van der Veyver⁴, Ronald Wapner², and Lyn S. Chitty^{1,5,*}

¹North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK

²Department of Obstetrics and Gynecology, Division of Reproductive Genetics, Columbia University, New York, NY, USA

³Department of Obstetrics and Gynecology, Division of Maternal Fetal Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

⁴Departments of Obstetrics and Gynecology and Molecular and Human Genetics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX, USA

⁵Genetics and Genomic Medicine, UCL Great Ormond Street Institute of Child Health, London, UK

Abstract

Prenatal genetic diagnosis provides information for pregnancy and perinatal decision-making and management. In several small series, prenatal whole exome sequencing (WES) approaches have identified genetic diagnoses when conventional tests (karyotype and microarray) were not diagnostic. Here, we review published prenatal WES studies and recent conference abstracts. Thirty-one studies were identified, with diagnostic rates in series of five or more fetuses varying between 6.2% and 80%. Differences in inclusion criteria and trio versus singleton approaches to sequencing largely account for the wide range of diagnostic rates. The data suggest that diagnostic yields will be greater in fetuses with multiple anomalies or in cases preselected following genetic review.

Beyond its ability to improve diagnostic rates, we explore the potential of WES to improve understanding of prenatal presentations of genetic disorders and lethal fetal syndromes. We discuss prenatal phenotyping limitations, counselling challenges regarding variants of uncertain significance, incidental and secondary findings, and technical problems in WES. We review the practical, ethical, social and economic issues that must be considered before prenatal WES could become part of routine testing. Finally, we reflect upon the potential future of prenatal genetic diagnosis, including a move towards whole genome sequencing and non-invasive whole exome and whole genome testing.

*Correspondence to: L. S. Chitty. l.chitty@ucl.ac.uk.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

INTRODUCTION

Fetal abnormalities are identified in 2%–5% of pregnancies and are responsible for 20% of perinatal deaths.^{1–4} Pregnancy outcome and prognosis vary according to the types of abnormalities detected, whether they are isolated or multiple and what the genetic aetiology may be. Currently, prenatal testing strategies include quantitative fluorescence polymerase chain reaction and fluorescence in situ hybridisation for rapid aneuploidy testing, G-banded karyotyping and chromosomal microarray analysis (CMA) for detection of copy number variation. These cytogenetic tests are limited by their resolution. Quantitative fluorescence polymerase chain reaction demonstrates trisomy in around 30% of dysmorphic fetuses, and karyotyping identifies pathogenic unbalanced chromosomal rearrangements in a further 5%. CMA finds additional pathogenic copy number variants in 3% to 6.5% of structurally abnormal fetuses with normal karyotypes.^{5,6} Using these tests in combination, an underlying genetic aetiology can be identified in up to 40% of dysmorphic fetuses, still leaving the majority of cases undiagnosed.

Next generation whole exome sequencing (WES) and whole genome sequencing (WGS) provide much greater resolution, down to the single base-pair level. For WGS, the genome (coding and non-coding regions) is sequenced without prior selection, whereas for WES, currently the more commonly used method for diagnostic applications, DNA regions containing the protein-coding exons, which make up 1% to 2% of the genome, but contain >85% of all disease-causing mutations, are first selectively captured before sequencing. In postnatal testing, WES studies have demonstrated a diagnostic yield of 25% to 30% in patients suspected to have a genetic condition undiagnosed using established methods.^{7,8} Prior to the adoption of next generation sequencing (NGS) techniques in prenatal diagnostics, the ability to accurately diagnose point mutations or small copy number changes in fetuses has been limited to a small number of genetic diseases with recognisable fetal phenotypes.⁹ Many monogenic disorders present prenatally with abnormalities detected by sonography and are likely to account for a significant proportion of cases undiagnosed using cytogenetic techniques, many of which may be detectable using WES.¹⁰ If a specific phenotype is known that suggests particular sets of monogenic disorders, more focused targeted gene panels, rather than WES, can be used. However, their prenatal application is limited by the more incomplete phenotypic ascertainment and because most were designed for postnatal use for phenotypes not all of which may present prenatally.

Prenatal testing to inform ongoing pregnancy management requires a rapid turnaround time as has been shown in the postnatal setting where WES and WGS have been successfully adopted for very rapid emergency genetic diagnoses on paediatric and neonatal intensive care units.¹¹

Here, we review the literature on prenatal WES for diagnosis of structurally abnormal fetuses. We discuss the promises and challenges of prenatal WES, and issues that must be considered before prenatal WES could become widely implemented. We also discuss future directions for prenatal genetic testing.

EXISTING PRENATAL WES STUDIES

A review of the literature using the PubMed tool was undertaken searching articles that reported WES or WGS on prenatal samples. A manual search for additional unpublished abstracts from prenatal and genetics conferences of relevant major societies was performed for the past 3 years (2014 up to May 2017). These included the American College of Medical Genetics and Genomics (ACMG), American Society of Human Genetics (ASHG), British Society of Genetic Medicine (BSGM), European Society of Human Genetics (ESHG), International Society for Prenatal Diagnosis (ISPD) and the Society for Maternal-Fetal Medicine (SMFM). The following keywords were used for the literature search: 'prenatal', 'fetal', 'whole exome sequencing' and 'whole genome sequencing'. Publications were included if they described genetic testing with WES or WGS on prenatal samples for fetal anomalies, either for ongoing pregnancies or termination of pregnancy. They were included only if they were in English, published between 2012 and 2017, studied humans and available in full text.

The literature search identified 205 articles. Titles and abstracts were examined, and 14 articles were included in this paper either as single case reports or larger case series. In addition, seventeen conference abstracts containing relevant data unpublished in the included articles were identified. The most recent abstract was selected when the same research was presented more than once. Table 1 includes details of all series of at least five cases, with further information about all individual cases and series provided in the Supporting Information Table S1.

Ten of the 31 reports describe single cases^{12–21} and there were five small series of two to four cases.^{82–86} The larger studies of five or more cases present a range of inclusion criteria including termination of pregnancy or fetal demise with fetal anomalies, euploid fetuses with structural anomalies on ultrasound, increased nuchal translucency (NT) >3.5 mm, single or multiple fetal anomalies (Table 1). A confounder in calculating the diagnostic rate is whether proband-only WES is performed, or trio WES, which has a reported higher diagnostic rate, both prenatally²² and in children.²³ Nearly half (7 of 16) of the larger series were complete trios for all cases,^{24–30} whilst the remainder included a combination of proband-only and trio WES.^{22,31–38} It is also important to note that not all studies reported a normal karyotype and/or CMA before proceeding to WES. However, even in studies with normal conventional testing, findings are highly variable.

The diagnostic rates ranged from 6.2%²⁹ to 80%³⁰ across the 16 studies with five or more fetuses tested.^{12–19,21,25,27,29,30,36–38} Studies on the two largest cohorts of unselected fetuses with any anomaly, including an isolated increased NT measurement, investigated by WES reported the lowest overall detection rates, but a higher diagnostic rate (14.3% and 16%) for fetuses with multiple anomalies compared with overall^{28,29} (Table 1). A genetic aetiology was only identified for 1/28 (3.6%) of fetuses with an isolated increased NT measurement or cystic hygroma.²⁸ The higher detection rates for fetuses with multiple anomalies in these early studies suggest that WES may be best targeted to fetuses with multiple structural anomalies. When testing single structural anomalies, a particular organ system may have a higher diagnostic yield. For example, Walkiewicz *et al.*³⁴ identified diagnoses for 3/6 (50%)

of fetuses with brain abnormalities compared with 8/28 (29%) with an anomaly not including the brain. Clearly, further research is required to determine how best to triage cases for WES.

At the other end of the spectrum, the study³⁰ with the highest diagnostic rate of 80% tested five trios with very strict entry criteria that included parental consanguinity, loss of heterozygosity on single nucleotide polymorphism array and fetal features highly suggestive of a syndrome for which multiple causative genes are known. All diagnostic rates that were 50% or greater were reported in smaller series of 15 or fewer cases^{12,18,19,30,37} where the inclusion criteria were unclear but likely to be highly selected for multiple anomalies.

The wide diagnostic rates revealed by these studies highlight the importance of considering a priori risk for single gene disorders, performing prior conventional testing and targeting trios before selecting patients for prenatal WES.

PROMISES

Diagnosis, informed decision-making and genetic counselling Results interpretation in the genomic era requires a multidisciplinary team approach comprising clinical scientists, clinical geneticists, genetic counsellors and specifically for prenatal diagnosis, fetal medicine specialists expert in prenatal dysmorphology. When fetal anomalies are detected, accurate prenatal diagnosis is valuable to provide information for reproductive decision-making, including pregnancy and perinatal management options.³⁹ Prenatal genetic diagnosis from a single comprehensive test can prevent the diagnostic odyssey that frequently occurs postnatally, potentially reducing the costs of serial testing, including molecular, imaging and other pathological investigations, some of which may be invasive, such as biopsies. An alternative option to prenatal WES is postnatal 'critical trio WES', which often happens in cases where prenatal WES could have helped, but was not performed because of financial constraints in some countries where insurance payments may be declined prenatally but allowable in the postnatal setting. A prenatal diagnosis may therefore reduce extended neonatal hospital stays whilst awaiting a diagnosis. Furthermore, diagnosing a lethal genetic condition before birth allows parents time to make choices about the pregnancy management, including termination within local legal limits, or discussion about palliative care before delivery. Identification of a genetic diagnosis also allows counselling about recurrence risks and can facilitate preparation for prenatal testing or pre-implantation genetic diagnosis in subsequent pregnancies.

Treatment

Prenatal diagnosis can reduce morbidity and mortality by guiding delivery plans and early treatment for fetuses with some genetic abnormalities. For example, pyruvate dehydrogenase deficiency, an inborn error of metabolism, can present during pregnancy with fetal brain abnormalities such as ventriculomegaly and brain dysgenesis.^{23,39–41} In this situation, diagnosis following prenatal WES could guide immediate postnatal treatment with management of acidosis and early introduction of a ketogenic diet with thiamine supplementation,^{42,43} resulting in an improved long-term outcome.

Furthermore, WES may be useful in situations where in utero fetal therapy is being considered. For example, fetal endotracheal occlusion may improve outcome for fetuses with congenital diaphragmatic hernia. However, genetic syndromes are relatively common in fetuses with congenital diaphragmatic hernia, not all of which will have additional sonographic findings to guide testing.⁴⁴ Similarly, in utero surgery for cardiac anomalies including hypoplastic left heart syndrome⁴⁵ is also now possible, but in Europe, nearly 20% of babies born with a heart anomaly had recognisable genetic syndromes.⁴⁶ Prenatal WES would offer additional and accurate prognostic information for selecting those patients for whom the risk–benefit balance is the most favourable for these invasive fetal procedures.

Identification of molecular genetic diagnoses prenatally may facilitate targeted therapy, with potential to improve the clinical outcome for the child. For example, postnatal mesenchymal stem cell transplantation in children with osteogenesis imperfecta (OI) increased skeletal mineralisation and growth velocity. Subsequently, in utero therapy for two fetuses diagnosed with OI has demonstrated a reduction in expected fractures and their sequelae.⁴⁷ A pan-European study plans to treat 15 fetuses with suspected OI in utero in the Boost Brittle Bones Before Birth (BOOSTB4) study to further investigate safety before embarking on a larger scale trial.⁴⁸ Many cases of OI arise *de novo* in pregnancy, and as this is a very heterogeneous condition, exome sequencing offers the only approach to rapid, comprehensive molecular prenatal diagnosis, which will be required prior to embarking on in utero therapy. With developments in gene and stem cell therapy, this type of treatment may increasingly become an option for other conditions.

Expanding prenatal phenotypes and improving understanding of human fetal development

Prenatal WES studies have been successful in identifying previously unrecognised prenatal phenotypes associated with known Mendelian disease genes, and in identifying novel candidate genes for human developmental disorders. For example, using WES to investigate fetuses from non-continuing pregnancies with sonographic abnormalities, a pathogenic variant in *KCTDI* causative of scalp ear nipple syndrome was identified in a terminated fetus with skin sloughing and hypoplastic nails noted on post-mortem examination.²⁷ Prenatal findings included anhydramnios and a complex heart defect; anomalies of the kidneys and heart defects have been reported previously only in postnatally diagnosed cases. A *de novo* truncating mutation in the *MAP4K4* gene was also found in a fetus with a complex cardiac defect, identifying a possible novel candidate gene for a human developmental disorder given its essential role in embryonic cardiac developmental in mouse models.

Prenatal WES and WGS studies also have the potential to improve understanding of lethal genetic disorders presenting with fetal abnormalities, where the full phenotype is often unknown. NGS approaches will facilitate gene discovery and may reveal Mendelian inheritance where previously missed. Using trio WES in a family with recurrent intrauterine growth restriction and multiple abnormalities, a new lethal ciliary disorder was identified when autosomal recessive truncating mutations in the *KIF14* gene that segregated with the phenotype were detected.¹⁴ Pathogenicity was supported by evidence from mouse models with autosomal recessive mutations in *KIF14*, which have a very similar phenotype.⁴⁹ It has

been suggested that cross-species phenotyping may be valuable in providing further evidence for causality of identified variants in genes involved in ultra-rare and lethal phenotypes.⁵⁰

PITFALLS

Pre-test and post-test counseling

Pre-test counselling for prenatal WES and WGS presents a challenge given the complexity of information to convey and the range of findings that may be revealed. Although genome-wide tests may have an improved ability to detect clinically significant findings compared with specific gene tests, they also increase the chance of detecting incidental findings, secondary findings and variants of uncertain significance.⁵¹ Although these issues are not novel, WES in the prenatal setting poses an ethical challenge given the potential to influence pregnancy decision-making based on identified variants. Pre-test counselling for prenatal WES needs to be detailed, delivered in an intelligible fashion to patients from all educational and cultural backgrounds. This is therefore best performed by trained genetic professionals. Counselling must emphasise that prenatal WES tests are new, still being evaluated and do not yet have well-defined clinical utility, sensitivity or specificity. Estimates about diagnostic rates should be conservative and ideally stratified according to the type and number of fetal anomalies detected. Post-test counselling should be informed by the multidisciplinary team, which should include clinical scientists, clinical geneticists and relevant clinical specialists. It needs to include the significance of any findings, details of conditions diagnosed including the prognosis and potential therapies. Specialist input will also be required from genetic counsellors and clinical geneticists to explain the inheritance and cascade investigations to the wider family if needed.

Secondary and incidental findings

Secondary findings are genetic variants, unrelated to the primary presentation, which may be reported if they are deemed medically actionable. For postnatal WES, the ACMG recommends that clinical laboratories actively seek out and report known and likely pathogenic variants in 59 genes, which largely predispose to cancer and cardiac disease⁵² and will be found in about 1% of the population.^{53,54} ACMG guidance suggests that counselling regarding secondary findings should be included during pre-test counselling, with an opt-out option, but elsewhere practice is determined on an individual study basis. Professional guidelines have been issued regarding predictive testing in children, with most recommending that unless testing has current medical benefit, it should be deferred until a child is old enough to make her/his own decision.^{55,56} However, recent ASHG guidance suggests that in some families predispositional testing for juniors may be reasonable, and these requests should be discussed individually.⁵⁷ These guidelines provide a useful basis for consideration of testing for secondary findings in the prenatal period.

Incidental findings are observations or results of potential clinical significance that are unexpectedly discovered and are unrelated to the purpose of the test.⁵⁸ Trio WES and WGS could also reveal unforeseen issues such as non-paternity or parental consanguinity. The chances of identifying incidental findings can be reduced by applying phenotype-specific

gene panels or targeted interpretation of sequence data. If NGS approaches are integrated into mainstream prenatal services, guidelines on reporting of incidental findings will be essential and should encompass the consent procedure. These could be in keeping with current ACMG guidelines on reporting in the postnatal setting,⁵³ on which there are high levels of concordance amongst specialists.⁵⁹

Interpretation of variants

Variants of uncertain significance are those for which pathogenicity is unclear. When reported to patients, they can cause significant anxiety and make decision-making challenging. There is currently no fetal variant database equivalent to postnatal databases like ClinVar⁶⁰ or the Human Gene Mutation Database,⁶¹ and there is no organised process for prenatal providers to determine the pathogenicity of genomic variants. Instead, providers rely on individual laboratory reports, which have high inter-rater variability⁶² or on individual literature searches, which can be cumbersome, time-consuming and limited in scope. Given the increasing calls to pursue WES/WGS for genetic diagnosis of fetal congenital anomalies and the paucity for most genes of systemically classified variants associated with specific fetal phenotypes, there is strong scientific premise and an urgent unmet need for a formal curation procedure for prenatal genomic variants. This should ideally be combined with the development of an international registry of the fetal phenotypes associated with (or caused by) these variants.

Fetal phenotyping

One of the major practical challenges of prenatal WES or WGS is the potential long turnaround time for these tests. Although detection of severe or lethal congenital disorders is becoming more frequent earlier in pregnancy as a result of first trimester scanning to measure NT and perform Down syndrome screening,^{63,64} most structural fetal anomalies are detected at the mid-pregnancy ultrasound scan between 18 and 22 weeks gestational age. This means that genetic testing must be completed quickly to inform decision-making during a pregnancy, requiring the DNA sequencing and data interpretation pipeline to be fast.³² Rapid turnaround of results from WES during ongoing pregnancies has been reported in one small study demonstrating a 50% diagnosis rate and turnaround time of 3 weeks.²⁶ Use of an analytical pipeline targeting gene panels specific to particular fetal abnormalities may also facilitate a faster turnaround time and reduce detection of incidental findings. For this to be achieved, phenotypes must be specific and well defined, which is particularly challenging prenatally. Hence, practically, WES may currently be the most efficient mechanism to achieve rapid diagnoses. As understanding of variants grows, it is possible that WES could help inform contents of future targeted gene panels. However, this will have to be weighed against parallel improvements in WES annotation.

Despite progress in prenatal ultrasound and magnetic resonance imaging, difficulties in fetal phenotyping make prenatal genotype–phenotype correlation more challenging than postnatal. The skill level of practitioners performing ultrasound can vary greatly and may cause both over- and under-diagnosing of fetal structural abnormalities. For these reasons, great caution must be exercised in interpreting variants not previously reported to avoid over-interpretation of potentially normal variants. In addition, subtle dysmorphic features of

many monogenic disorders cannot be determined using fetal ultrasound. Furthermore, variable presentations of fetal disorders, incomplete penetrance and appearance of ultrasound abnormalities only at late gestational ages can complicate interpretation. Some phenotypes are impossible to determine from prenatal imaging, such as developmental delay and intellectual disability. Therefore, it is harder to identify the most appropriate candidate genes or apply the correct gene panels when that phenotypic detail is not available. When pregnancies are ended, postnatal examination by experts in dysmorphology and fetal autopsy at post-mortem can be very useful to refine the phenotype and target genes for investigation more specifically.²⁷

PRACTICAL PROBLEMS IN WES

WES is challenged by the incomplete coverage of relevant genes, which may compromise the interpretation of exome sequences. The limited timeframe required for prenatal diagnostic reporting means that extending coverage of candidate genes by alternative methods of sequencing is not always feasible. Although depth of coverage from exome sequencing is likely to improve, some regions of the genome are practically difficult to capture, for example, because of repetitive sequences and high GC content.⁶⁵ Genes within families of highly homologous genes or those that have a pseudogene are also difficult to sequence.

The emphasis of NGS strategies in fetal diagnostics has been on WES rather than WGS to date. This relates to its lower costs, need for greater speed and ease in data handling due to our limited ability to interpret intronic variants. However, WES is not currently able to reliably detect all types of genetic variation such as copy number changes, low level mosaicism, aneuploidy, structural chromosome rearrangements or trinucleotide repeat expansions and cannot detect variants in non-coding regions. Therefore, conventional testing options such as microarrays should still be considered in parallel to prenatal WES, especially when there is a clinical suspicion for known prenatal presentations of genetic syndromes.

IMPLEMENTATION

Guidelines

The ACMG recommends that WES be considered when specific genetic tests for a phenotype fail to determine a diagnosis in a fetus with multiple anomalies suggestive of a genetic disorder.⁶⁶ The American College of Obstetrics and Gynecology does not recommend the routine use of WES but states that if considered it should be ordered after consultation with a clinical geneticist because of the complexity of analysis.⁶⁷ The Joint UK Committee on Genomics in Medicine is currently conducting 'new genomic technologies in pregnancy' workshops as a follow on to their 'recommendations for the use of CMA in pregnancy'.⁶⁸ The outcome of these workshops is awaiting publication and dissemination.

Costs

The cost effectiveness of prenatal WES has not been formally evaluated. A useful comparison from the postnatal setting includes a budget impact model comparing WES with

traditional care pathways in the investigation of children with neurodevelopmental disorders of unknown genetic aetiology.⁶⁹ They compared traditional care pathways with those incorporating WES, using an average cost of exome sequencing of \$2439. Using WES, after normal CMA and fragile X testing gave a cost saving of \$1.33 million for a planned size of one million cases. In the prenatal setting, trio WES is likely to be required in most situations to deliver the fast turnaround times required for results to be useful in informing pregnancy management, further increasing the costs.

Additional costs generated from WES studies must also be considered. Despite dramatic decreases in costs of generating DNA sequences, the costs of variant interpretation are unlikely to fall as quickly given the manpower required.⁷⁰ The infrastructure requirements to store and utilise genomic sequence findings are significant.⁷¹ Identification of secondary findings requires initiation of confirmatory tests, targeted treatments and follow-up screening. Implementation of reinterpretation or re-analysis of previously studied data stored from proband or trio samples in light of emerging reclassified or newly identified variants will require strategies with associated costs that have yet to be addressed. Lastly, the cost for more in-depth genetic counselling by trained genetic counsellors, clinical geneticists and maternal fetal medicine and other relevant specialists with genetic expertise, all of which are in short supply in many parts of the world, should also be taken into account.

Societal and ethical issues

Ethical issues surrounding prenatal WES have been reviewed extensively.⁷² The identification of genetic findings before birth denies the child of their autonomy to decide whether they wish to know this information.⁷³ Based on genetic information, parents may choose to terminate pregnancies for reasons that some find inappropriate or objectionable or may treat and raise children differently once they are born. This may present a significant psychological burden to both parents and children.⁵⁷ However, for some families and conditions, not knowing the cause of abnormalities during pregnancy could present an equally significant burden, so these two scenarios must be weighed up carefully for each family. Further debate is required to determine whether withholding information regarding disease predisposition in the prenatal setting is required.

Patient and provider attitudes

A survey of 186 expectant parents in the United States found that 83% thought that WES should be offered in the prenatal setting.⁷⁴ An in-depth study assessing perspectives of 15 women with non-continuing anomalous pregnancies found that women in the highest socio-economic group had significantly higher pre-test genomic knowledge than their lower income counterparts.²⁷ They also found that women had high hopes and expectations of WES, despite having been advised that the diagnostic yield was approximately 30%. This suggests that highly tailored genetic counselling or extra decision aids may be useful to improve patient understanding of sequencing.

Focus groups in the UK have explored views on prenatal WES from clinicians, scientists, genetic counsellors and patients.⁷⁵ Participants representing patient groups/charities expressed the view that women undergoing prenatal WES would want to be told all possible

information, in contrast to clinicians who were more cautious about disclosure of incidental or uncertain findings. Patient representative groups also would want reinterpretation of results over time, whilst clinicians felt that interpretation should be performed at the point of testing only. A survey undertaken by 1114 members of the American College of Obstetrics and Gynecology about the ethical issues surrounding prenatal WGS and their preparedness for counselling patients on its use revealed undercurrents of concern regarding this new technology.⁷⁶ The obstetricians/gynaecologists were most concerned that ordering an expansive test could lead to overtreatment, higher cost of care and may increase parental anxiety. They were more worried about receiving genomic results that could reveal non-medical traits such as musical ability or height, or learning disability, than results for diseases that often cause infant death. Given the complexities in counselling, the study emphasised the importance of extensive health profession education before prenatal WGS could be integrated into clinical care, including support from and collaboration with genetic counsellors.

THE FUTURE

Emphasis may switch from prenatal WES approaches towards WGS as the costs of sequencing fall, and bioinformatic and analytic capabilities improve. This will improve diagnostic capabilities because of improved ability to detect a wider range of genomic abnormalities, including non-coding variants, copy number changes and larger rearrangements. However, this will also increase the challenges posed by identification of incidental findings.

The use of cell-free DNA testing in aneuploidy screening and non-invasive prenatal diagnosis for a growing number of monogenic disorders is transforming prenatal diagnosis. Non-invasive prenatal diagnosis is already in routine use in the UK using NGS approaches in families at high risk of a genetic disorder, allowing around 30% of all molecular prenatal diagnosis to be performed using this safer approach.⁷⁷ Furthermore, proof-of-principle studies have shown that it is technically feasible to sequence the entire fetal genome using cell-free fetal DNA, although this is not yet achievable in a timely or cost effective way.^{78,79} Genome-wide sequencing of cell-free fetal DNA is challenged by incomplete coverage. This may result in high cumulative false positive rates, leading to unnecessary diagnostic procedures, and high false negative rates, resulting in missed genetic diagnoses and false reassurance to providers and parents.⁸⁰ However, as WES and WGS become more refined and costs fall, it may provide earlier diagnosis, reduce the need for invasive testing with the associated risk of miscarriage and detect Mendelian disorders in families who would not otherwise have been offered prenatal testing.⁸¹ Non-invasive fetal WGS could also augment or replace current approaches to newborn screening, facilitating earlier diagnosis of a wider range of conditions. This could improve prenatal care, pregnancy and delivery management and/or postnatal intervention.

CONCLUSION

In this review, we have explored the potential for improved prenatal diagnosis using WES, and the opportunity for improved understanding of prenatal genetic disorders. Although

there is relatively little data available, most of which is highly selected, it is clear that WES improves prenatal diagnosis in euploid fetuses with abnormal ultrasound findings. However, the diverse range of diagnoses made limits the generalisability of conclusions about diagnostic success rates from prenatal WES approaches. The data presented here suggest that diagnostic yields will be greater in fetuses with multiple anomalies or in cases preselected following genetic review, but we must await publication of larger cohorts of prenatal cases to determine in which subgroups WES may have the greatest clinical utility.

This technology offered in the prenatal setting comes with many challenges, including practical, ethical and economic issues. Further research is required to improve the evidence base of what is technically possible and what is clinically beneficial before WES could become part of routine prenatal testing. What is clear is that implementation must be accompanied by health professional education to enable better access to the expert genetic counselling that will be required both before and after testing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Update on overall prevalence of major birth defects – Atlanta, Georgia 1978–2005. MMWR Morb Mortal Wkly Rep. 2008; 57:1–5. [PubMed: 18185492]
2. Mathews TJ MM, MacDorman F, Thoma ME. Infant mortality statistics from the 2013 period linked birth/infant death data set. National Vital Statistics Reports [Internet]. 2015; 64(9):1–30. 08/03/2017. Available from: <https://www.cdc.gov/ncbddd/birthdefects/data.html>.
3. Boyd PA, Tonks AM, Rankin J, et al. Monitoring the prenatal detection of structural fetal congenital anomalies in England and Wales: register-based study. J Med Screen. 2011; 18:2–7. [PubMed: 21536809]
4. Calzolari E, Barisic I, Loane M, et al. Epidemiology of multiple congenital anomalies in Europe: a EUROCAT population-based registry study. Birth Defects Res A Clin Mol Teratol. 2014; 100:270–6. [PubMed: 24723551]
5. Wapner RJ, Martin CL, Levy B, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med. 2012; 367:2175–84. [PubMed: 23215555]
6. Callaway JL, Shaffer LG, Chitty LS, et al. The clinical utility of microarray technologies applied to prenatal cytogenetics in the presence of a normal conventional karyotype: a review of the literature. Prenat Diagn. 2013; 33:1119–23. [PubMed: 23983223]
7. Yang Y, Muzny DM, Reid JG, et al. Clinical whole-exome sequencing for the diagnosis of Mendelian disorders. N Engl J Med. 2013; 369:1502–11. [PubMed: 24088041]
8. Deciphering Developmental Disorders Study. Large-scale discovery of novel genetic causes of developmental disorders. Nature. 2015; 519:223–8. [PubMed: 25533962]

9. Chitty LS, Mason S, Barrett AN, et al. Non-invasive prenatal diagnosis of achondroplasia and thanatophoric dysplasia: next-generation sequencing allows for a safer, more accurate, and comprehensive approach. *Prenat Diagn.* 2015; 35:656–62. [PubMed: 25728633]
10. Medeira A, Norman A, Haslam J, et al. Examination of fetuses after induced abortion for fetal abnormality – a follow-up study. *Prenat Diagn.* 1994; 14:381–5. [PubMed: 8084858]
11. Willig LK, Petrikin JE, Smith LD, et al. Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings. *Lancet Respir Med.* 2015; 3:377–87. [PubMed: 25937001]
12. Talkowski ME, Ordulu Z, Pillalamarri V, et al. Clinical diagnosis by whole-genome sequencing of a prenatal sample. *New Engl J Med.* 2012; 367:2226–32. [PubMed: 23215558]
13. Shamseldin HE, Swaid A, Alkuraya FS. Lifting the lid on unborn lethal Mendelian phenotypes through exome sequencing. *Gen Med.* 2013; 15:307–9.
14. Filges I, Nosova E, Bruder E, et al. Exome sequencing identifies mutations in KIF14 as a novel cause of an autosomal recessive lethal fetal ciliopathy phenotype. *Clin Genet.* 2014; 86:220–8. [PubMed: 24128419]
15. Drury S, Boustred C, Tekman M, et al. A novel homozygous ERCC5 truncating mutation in a family with prenatal arthrogryposis – further evidence of genotype-phenotype correlation. *Am J Med Genet Part A.* 2014; 164A:1777–83. [PubMed: 24700531]
16. Wilbe M, Ekvall S, Eurenium K, et al. MuSK: a new target for lethal fetal akinesia deformation sequence (FADS). *J Med Genet.* 2015; 52:195–202. [PubMed: 25612909]
17. Kan A, Au PKC, Li M, et al. Exome sequencing on a family with 3 pregnancies affected by central nervous system malformation identified a novel stop mutation in WDR81. *Prenat Diagn.* 2015; 35(Suppl 1):71.
18. Casey J, Flood K, Ennis S, et al. Intra-familial variability associated with recessive RYR1 mutation diagnosed prenatally by exome sequencing. *Prenat Diagn.* 2016; 36:1020–6. [PubMed: 27616680]
19. Romagnoli MPE, Palombo F, Bonara E, Seri M. RYR1 related congenital myopathy in two sib fetuses conceived through artificial insemination with donor sperm (AID). *Eur J Hum Genet.* 2016; 24(E-Suppl 1):26.
20. Alamillo, C., Kobari, J., Powis, Z., et al. Clinical Diagnostic Exome Sequencing Identified Compound Heterozygous ASPM Gene Alterations in a Fetus with Marked Microcephaly; Presented at ACMG Annual Clinical Genetics Meeting; Tampa, Florida. 2016. https://acmg.expoplanner.com/index.cfm?do=expomap.sess&event_id=7&session_id=1505
21. Kooper AHW, Smeets D, Lugtenberg D, et al. Prenatal diagnosis of a rare, autosomal recessive disorder by combining genome wide array analysis and whole exome sequencing. *Prenat Diagn.* 2016; 36(Suppl 1):76.
22. Yates, CL., Monaghan, KG., Copenheaver, D., et al. Whole-exome sequencing on deceased fetuses with ultrasound anomalies: expanding our knowledge of genetic disease during fetal development. *Genet Med.* 2017. <https://doi.org/10.1038/gim.2017.31>. [Epub ahead of print]
23. Retterer K, Juusola J, Cho MT, et al. Clinical application of whole-exome sequencing across clinical indications. *Genet Med.* 2016; 18:696–704. [PubMed: 26633542]
24. Carss KJ, Hillman SC, Parthiban V, et al. Exome sequencing improves genetic diagnosis of structural fetal abnormalities revealed by ultrasound. *Hum Mol Genet.* 2014; 23:3269–77. [PubMed: 24476948]
25. Alamillo CL, Powis Z, Farwell K, et al. Exome sequencing positively identified relevant alterations in more than half of cases with an indication of prenatal ultrasound anomalies. *Prenat Diagn.* 2015; 35:1073–8. [PubMed: 26147564]
26. Drury SES, Boustred C, Jenkins L, Chitty L. Prenatal diagnosis of sonographic abnormalities using rapid whole exome sequencing. *Prenat Diagn.* 2016; 36(Suppl 1):51.
27. Vora NL, Brandt A, Strande N, et al. Prenatal exome sequencing in anomalous fetuses: new opportunities and challenges. *Genet Med.* 2017 in press.
28. Wapner RPS, Brennan K, Bier L, et al. Whole exome sequencing in the evaluation of fetal structural anomalies: a prospective study of sequential patients. *Am J Obstet Gynecol.* 2017; 216:S5–S6. [http://www.ajog.org/article/S0002-9378\(16\)30988-7/fulltext](http://www.ajog.org/article/S0002-9378(16)30988-7/fulltext).

29. McMullan, DJ., Eberhardt, R., Rinck, G., et al. European Society of Human Genetics Copenhagen. Denmark: 2017. Exome Sequencing of 406 Parental/Fetal Trios with Structural Abnormalities Revealed by Ultrasound in the UK Prenatal Assessment of Genomes and Exomes (PAGE) Project.
30. Yadava SM, Ashkinadze E. 125: Whole exome sequencing (WES) in prenatal diagnosis for carefully selected cases. *Am J Obstet Gynecol.* 2017; 216:S87–S88. [http://www.ajog.org/article/S0002-9378\(16\)31008-0/fulltext](http://www.ajog.org/article/S0002-9378(16)31008-0/fulltext).
31. Yang Y, Muzny DM, Xia F, et al. Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA.* 2014; 312:1870–9. [PubMed: 25326635]
32. Drury S, Williams H, Trump N, et al. Exome sequencing for prenatal diagnosis of fetuses with sonographic abnormalities. *Prenat Diagn.* 2015; 35:1010–7. [PubMed: 26275891]
33. Pangalos C, Hagnefelt B, Lilakos K, Konialis C. First applications of a targeted exome sequencing approach in fetuses with ultrasound abnormalities reveals an important fraction of cases with associated gene defects. *PeerJ.* 2016; 4:e1955. [PubMed: 27168972]
34. Walkiewicz, M., Braxton, A., Liu, P., Xia, PF, Bi, W., Xiao, R., et al. The utility of whole exome sequencing in prenatal diagnosis; Presented at the American Society of Human Genetics Annual Meeting; Vancouver. 2016. <https://ep70.eventpilot.us/web/planner.php?id=ASHG16>
35. Barnett, CP., Byrne, A., Moore, L., Khong, Y., Manton, N., Lipsett, J., et al. The genomic autopsy: using whole exome and whole genome sequencing to solve complex fetal and neonatal presentations; Presented at the American Society of Human Genetics Annual Meeting; Vancouver. 2016. <https://ep70.eventpilot.us/web/planner.php?id=ASHG16>
36. Ryan, E., Friedman, B., Haskins, A., Barbar, R., Nelson, Z., Al-Musafri, F., et al. Whole exome sequencing in 129 fetuses with abnormal ultrasound findings; Presented at ACMG Annual Clinical Genetics Meeting; Phoenix, Arizona. 2017. https://acmg.expoplanner.com/index.cfm?do=expomap.sess&event_id=8&session_id=5012
37. Sá, J., Melo, F., Tarelho, A., et al. Broad multi-gene panel or whole exome sequencing in malformed fetuses reveals eight definitive and one likely diagnoses in fifteen studied fetus, in prenatal setting. *ESHG.* 2017. <https://2017.eshg.org/index.php/abstracts-2/online-planner-abstract-search/>
38. Joset, P., Wisser, J., Niedrist, D., et al. Mendeliome and whole exome sequencing in 60 fetuses with abnormal ultrasound revealed a diagnostic yield of 30%. *ESHG.* 2017. <https://2017.eshg.org/index.php/abstracts-2/online-planner-abstract-search/>
39. Skirton H, Goldsmith L, Jackson L, et al. Offering prenatal diagnostic tests: European guidelines for clinical practice. *Eur J Hum Genet.* 2014; 22:580–6. [PubMed: 24022298]
40. Illsinger S, Das AM. Impact of selected inborn errors of metabolism on prenatal and neonatal development. *IUBMB Life.* 2010; 62:403–13. [PubMed: 20503433]
41. Natarajan N, Tully HM, Chapman T. Prenatal presentation of pyruvate dehydrogenase complex deficiency. *Pediatr Radiol.* 2016; 46:1354–7. [PubMed: 27026023]
42. Robinson JN, Norwitz ER, Mulkern R, et al. Prenatal diagnosis of pyruvate dehydrogenase deficiency using magnetic resonance imaging. *Prenat Diagn.* 2001; 21:1053–6. [PubMed: 11746163]
43. Sofou K, Dahlin M, Hallbook T, et al. Ketogenic diet in pyruvate dehydrogenase complex deficiency: short- and long-term outcomes. *J Inherit Metab Dis.* 2017; 40:237–45. [PubMed: 28101805]
44. McGivern MR, Best KE, Rankin J, et al. Epidemiology of congenital diaphragmatic hernia in Europe: a register-based study. *Arch Dis Child Fetal Neonatal Ed.* 2015; 100:F137–F144. [PubMed: 25411443]
45. Arzt W, Tulzer G. Fetal surgery for cardiac lesions. *Prenat Diagn.* 2011; 31:695–8. [PubMed: 21671460]
46. Stoll C, Clementi M. Prenatal diagnosis of dysmorphic syndromes by routine fetal ultrasound examination across Europe. *Ultrasound Obstet Gynecol.* 2003; 21:543–51. [PubMed: 12808670]
47. Westgren M, Götherström C. Stem cell transplantation before birth – a realistic option for treatment of osteogenesis imperfecta? *Prenat Diagn.* 2015; 35:827–32. [PubMed: 25962526]

48. Chitty LS, David AL, Gottschalk I, et al. BOOSTB4: a clinical study to determine safety and efficacy of pre- and/or postnatal stem cell transplantation for treatment of osteogenesis imperfecta. *Ultrasound Obstet Gynecol.* 2016; 48:356.
49. Fujikura K, Setsu T, Tanigaki K, et al. Kif14 mutation causes severe brain malformation and hypomyelination. *PLoS One.* 2013; 8:e53490. [PubMed: 23308235]
50. Filges I, Friedman JM. Exome sequencing for gene discovery in lethal fetal disorders – harnessing the value of extreme phenotypes. *Prenat Diagn.* 2015; 35:1005–9. [PubMed: 25046514]
51. Westerfield L, Darilek S, van den Veyver IB. Counseling challenges with variants of uncertain significance and incidental findings in prenatal genetic screening and diagnosis. *J Clin Med.* 2014; 3:1018–32. [PubMed: 26237491]
52. Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med.* 2017; 19:249–55. [PubMed: 27854360]
53. Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med.* 2013; 15:565–74. [PubMed: 23788249]
54. Johnston JJ, Rubinstein WS, Facio FM, et al. Secondary variants in individuals undergoing exome sequencing: screening of 572 individuals identifies high-penetrance mutations in cancer-susceptibility genes. *Am J Hum Genet.* 2012; 91:97–108. [PubMed: 22703879]
55. Ross LF, Saal HM, David KL, Anderson RR. Technical report: ethical and policy issues in genetic testing and screening of children. *Genet Med.* 2013; 15:234–45. [PubMed: 23429433]
56. Borry P, Evers-Kiebooms G, Cornel MC, et al. Genetic testing in asymptomatic minors: background considerations towards ESHG recommendations. *Eur J Hum Genet.* 2009; 17:711–19. [PubMed: 19277061]
57. Botkin JR, Belmont JW, Berg JS, et al. Points to consider: ethical, legal, and psychosocial implications of genetic testing in children and adolescents. *Am J Hum Genet.* 2015; 97:6–21. [PubMed: 26140447]
58. Kearney HM, Thorland EC, Brown KK, et al. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. *Genet Med.* 2011; 13:680–5. [PubMed: 21681106]
59. Green RC, Berg JS, Berry GT, et al. Exploring concordance and discordance for return of incidental findings from clinical sequencing. *Genet Med medicine.* 2012; 14:405–10.
60. Landrum MJ, Lee JM, Benson M, et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res.* 2016; 44(D1):D862–D868. [PubMed: 26582918]
61. Stenson PD, Mort M, Ball EV, et al. The human gene mutation database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Hum Genet.* 2017; 136(6):665–77. [PubMed: 28349240]
62. MacArthur DG, Manolio TA, Dimmock DP, et al. Guidelines for investigating causality of sequence variants in human disease. *Nature.* 2014; 508:469–76. [PubMed: 24759409]
63. Blaas HG. Detection of structural abnormalities in the first trimester using ultrasound. *Best Pract Res Clin Obstet Gynaecol.* 2014; 28:341–53. [PubMed: 24355991]
64. Cavoretto P, Giorgione V, Cipriani S, et al. Nuchal translucency measurement, free β -hCG and PAPP-A concentrations in IVF/ICSI pregnancies: systematic review and meta-analysis. *Prenat Diagn.* 2017; 37(6):540–55. [PubMed: 28419502]
65. Benjamini Y, Speed TP. Summarizing and correcting the GC content bias in high-throughput sequencing. *Nucleic Acids Res.* 2012; 40(10):e72. [PubMed: 22323520]
66. ACMG. Board of Directors Points to consider for informed consent for genome/exome sequencing. *Genet Med.* 2013; 15:748–9. [PubMed: 23970068]
67. Committee Opinion no. 682. Microarrays and next-generation sequencing technology: the use of advanced genetic diagnostic tools. *Obstet Gynecology.* 2016; 128:1462–3.
68. Gardiner C, WD., Kilby, M., Kerr, B. Recommendations for the use of chromosome microarray in pregnancy. 2015. 16/12/2016. Available from: http://www.bsgm.org.uk/media/956141/g144_useofcmmapregnancy_jun15.pdf

69. Sabatini LM, Mathews C, Ptak D, et al. Genomic sequencing procedure microcosting analysis and health economic cost-impact analysis: a report of the Association for Molecular Pathology. *J Mol Diagn*. 2016; 18:319–28. [PubMed: 27080370]
70. Beckmann JS. Can we afford to sequence every newborn baby's genome? *Hum Mutat*. 2015; 36(3):283–6. [PubMed: 25546530]
71. Sboner A, Mu XJ, Greenbaum D, et al. The real cost of sequencing: higher than you think! *Genome Biol*. 2011; 12:125. [PubMed: 21867570]
72. Horn R, Parker M. Opening Pandora's box: ethical issues in prenatal whole genome and exome sequencing. *Prenat Diagn*. 2017 in press.
73. Yurkiewicz IR, Korf BR, Lehmann LS. Prenatal whole-genome sequencing – is the quest to know a fetus's future ethical? *N Engl J Med*. 2014; 370:195–7. [PubMed: 24428465]
74. Kalynchuk EJ, Althouse A, Parker LS, et al. Prenatal whole-exome sequencing: parental attitudes. *Prenat Diagn*. 2015; 35:1030–6. [PubMed: 26151551]
75. Quinlan-Jones E, Kilby MD, Greenfield S, et al. Prenatal whole exome sequencing: the views of clinicians, scientists, genetic counsellors and patient representatives. *Prenat Diagn*. 2016; 36:935–41. [PubMed: 27550507]
76. Bayefsky MJ, White A, Wakim P, et al. Views of American OB/GYNs on the ethics of prenatal whole-genome sequencing. *Prenat Diagn*. 2016; 36:1250–6. [PubMed: 27862072]
77. Drury S, Mason S, McKay F, et al. Implementing non-invasive prenatal diagnosis (NIPD) in a National Health Service Laboratory; from dominant to recessive disorders. *Adv Exp Med Biol*. 2016; 924:71–75. [PubMed: 27753022]
78. Fan HC, Gu W, Wang J, et al. Non-invasive prenatal measurement of the fetal genome. *Nature*. 2012; 487(7407):320–4. [PubMed: 22763444]
79. Kitzman JO, Snyder MW, Ventura M, et al. Noninvasive whole-genome sequencing of a human fetus. *Sci Transl Med*. 2012; 4(137):137ra76.
80. Van den Veyver IB. Recent advances in prenatal genetic screening and testing. *F1000Research*. 2016; 5:2591. [PubMed: 27853526]
81. Tabor HK, Murray JC, Gammill HS, Kitzman JO, Snyder MW, Ventura M, et al. Non-invasive fetal genome sequencing: opportunities and challenges. *Am J Med Genet*. 2012; 158 A(10):2382–4.
82. Yang Y, Muzny DM, Reid JG, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *New Engl J Med*. 2013; 369:1502–11. [PubMed: 24088041]
83. Aggarwal STA, Das Bhowmik A, Dalal A. From fetal dysmorphology to exome sequencing: the diagnostic performance for making a perinatal genetic diagnosis. *Prenat Diagn*. 2016; 36(Suppl 1): 19.
84. Black HA, Parry D, Atanur SS, et al. De novo mutations in autosomal recessive congenital malformation. *Genet Med*. 2016; 18:1325–6. [PubMed: 27280866]
85. Pinto AM, Imperatore V, Bianciardi L, et al. Combined ultrasound and exome sequencing approach recognizes Opitz G/BBB syndrome in two malformed fetuses. *Clin Dysmorphol*. 2017; 26:18–25. [PubMed: 27749392]
86. Gijbbers, ACJ., Smeets, D., Pfundt, R., et al. Severe ultrasound abnormalities: prenatal whole exome sequencing to unravel a diagnosis before birth. *ESHG*. 2017. <https://2017.eshg.org/index.php/abstracts-2/online-planner-abstract-search/>. Accessed 08/06/2017

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Using established cytogenetic and molecular techniques, prenatal genetic diagnosis is only possible in around 40% of dysmorphic fetuses.
- Several small prenatal whole exome sequencing (WES) studies have successfully identified genetic diagnoses in fetuses with structural abnormalities when standard genetic testing was not diagnostic.
- Although WES improves detection of clinically important variants, it also increases detection of incidental findings and variants of uncertain significance, which makes interpretation and counselling challenging.

WHAT DOES THIS STUDY ADD?

- Our literature review identified a wide range of diagnosis rates, varying with inclusion criteria.
- Prenatal WES improves diagnostic rates and understanding of genetic disorders in the prenatal period.
- We discuss the challenges of prenatal WES including difficulties of fetal phenotyping, counselling and practical issues such as turnaround time, variant interpretation and cost.
- We highlight the ethical, social and economic factors requiring consideration before widespread implementation of prenatal WES.

Table 1
Series of at least five cases of fetal exome sequencing from published articles and conference abstracts

Reference	No. cases	Inclusion criteria	Method	Positive/definite diagnoses	Potentially pathogenic variants	Comments
Yang <i>et al.</i> ³¹	11	Terminated anomalous fetuses	Trio WES if possible	6/11 (54.5%)		Neurological findings increased diagnostic yield
Cuss <i>et al.</i> ²⁴	30	Euploid fetuses and neonates with prenatal anomalies	Trio WES	3/30 (10%)	5/30 (16.7%)	
Drury <i>et al.</i> ³²	24	Fetuses with raised NT >3.5 mm and/or other sonographic abnormalities with normal karyotype and/or CMA	14 singleton WES 10 trio WES	Overall: 5/24 (20.8%); Trios: 3/10 (30%), singletons: 2/14 (14.3%)	1/24 (4.2%)	2/24 (17%); conditions identified unrelated to sonographic findings
Alamillo <i>et al.</i> ²⁵	7	Fetal demise or termination of pregnancy, with multiple congenital abnormalities detected on ultrasound	Trio WES	3/7 (42.9%)	1/7 (14.3%)	6/7 cases; recurrent affected pregnancies in families
Pangalos <i>et al.</i> ³³	14	Fetuses with sonographic abnormalities and normal CMA: 11 ongoing and 3 terminated pregnancies	Singleton WES ‘Fetalis’ algorithm targeting 758 genes	Overall: 6/14 (42.9%); Terminated fetuses 3/3 (100%), ongoing pregnancies 3/11 (27.3%)		1/14 (7%); condition identified unrelated to sonographic findings
^a Walkiewicz <i>et al.</i> ³⁴	61	Selected fetuses with ultrasound anomalies and normal karyotype and CMA	40 Singleton WES 21 Trio WES	Overall: 20/61 (32.8%); Trios: 8/21 (38.1%), singletons: 12/39 (30.8%)		Results available in a 3- week timeframe
^a Drury <i>et al.</i> ²⁶	6	Fetuses with sonographic abnormalities	Trio WES	3/6 (50%)		
^a Barnett <i>et al.</i> ³⁵	8	Fetuses with sonographic abnormalities resulting in termination of pregnancy, in utero or neonatal death; prioritised consanguinity (singletons), multiple malformations and unexplained fetal/newborn death (trios)	Mixed singleton and trio WES and WGS	Overall: 4/8 (50%). Two known disease genes, two novel disease genes		
Yates <i>et al.</i> ²²	84	Fetal demise or termination of pregnancy	51 Trio/quad WES 33 singleton/duo WES	17/84 (20.2%)	38/84 (45.2%)	Diagnostic yield for trios 24% compared with 14% for singletons
Vora <i>et al.</i> ²⁷	15	Fetuses with multiple abnormalities and normal karyotype and CMA +/- specific gene sequencing	Trio WES	7/15 (46.7%); fetuses with multiple anomalies 5/35 (14.3%)	1/15 (6.7%)	1/15 (6.7%); medically actionable secondary finding identified

Reference	No. cases	Inclusion criteria	Method	Positive/definite diagnoses	Potentially pathogenic variants	Comments
^a Wapner <i>et al.</i> ²⁸	168	Sequential pregnancies with fetal abnormalities or increased NT >3.5 mm with normal karyotype and CMA	Trio WES	13/168 (7.7%)	30/168 (17.9%)	
^a Ryan <i>et al.</i> ³⁶	129	Fetuses with sonographic abnormalities	Trio and other family combination WES	Overall: 32/129 (25%); trios 21/78 (26.9%)	57/129 (44.2%) probable diagnosis 12/129 (9.3%) reportable variant in candidate gene	2/129 (1.6%): ACMG medically actionable secondary findings
^a McMullan <i>et al.</i> ²⁹	259	Unselected fetuses with sonographic abnormalities with normal qPCR and CMA	Trio WES	Overall: 16/259 (6.2%) Multisystem phenotypes: 16%		Diagnostic yield varies by phenotypic class
^a Yadava <i>et al.</i> ³⁰	5	Selected fetuses with sonographic abnormalities with normal karyotype and CMA, and consanguinity, loss of heterozygosity on SNP array, and features highly suggestive of a genetic syndrome	Trio WES	4/5 (80%)		1/5 (20%); medically actionable secondary finding detected
^a Sa <i>et al.</i> ³⁷	15	Sonographic abnormality and medical termination of pregnancy, or fetal death with multiple anomalies confirmed at autopsy	13/15: clinical exome (4813 genes). 2/15: trio WES	8/15 (53.3%)	1/15 (6.7%)	Only one of the identified diagnoses was previously suspected in the sonogram
^a Joset <i>et al.</i> ³⁸	60	Fetuses with various malformations	'Mendeliome' (panel of 3070 genes) and WES. Unclear whether singleton or trio	18/60 (30%)		2/60 (3.3%); new disease-causing candidate genes. In urgent cases, turnaround time was 2 weeks

^a It denotes conference abstract.