The role of next-generation sequencing in the investigation of ultrasound-identified fetal structural anomalies

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Fetal structural anomalies have an impact on fetal mortality and morbidity. Next-generation sequencing (NGS) may be incorporated into clinical pathways for investigation of paediatric morbidity but can also be used to delineate the prognosis of fetal anomalies. This paper reviews the role of NGS in the investigation of fetal malformations, the literature defining the clinical utility, the technique most commonly used and potential promise and challenges for implementation into clinical practice. Prospective case selection with informative pre-test counselling by multidisciplinary teams is imperative. Regulated laboratory sequencing, bioinformatic pathways with potential variant identification and conservative matching with the phenotype is important.

Keywords Exome, fetus, genome, sequencing, structural abnormality.

Tweetable abstract Prenatal exome sequencing in fetal structural anomalies yields diagnostic information in up to 20% of cases.

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Introduction

Prenatal ultrasound is established as a screening tool in obstetrics and with increasingly high resolution identifies fetal structural abnormalities in approximately 5% of pregnancies.[1] However, the overall prognosis and long-term outcome is often variable and dependent upon: (i) the type of principal fetal abnormality, (ii) whether there are additional structural abnormalities and (iii) whether there is an underlying global fetal pathology in the form of a chromosome or gene anomaly. The use of dysmorphology principles may be used to determine the underlying cause from the nature and patterns of fetal anomalies.[2] Indeed, when fetal abnormalities are identified, further prospective evaluation frequently includes invasive testing to detect whole chromosome aneuploidies (using quantitative fluorescence-polymerase chain reaction [QF PCR]) and chromosomal microarray analysis (CMA) to identify submicroscopic chromosomal anomalies, collectively referred to as 'copy number variants' (CNVs).

[Correction added on 15 March 2021, after online publication: The copyright was changed.]

Overall, QF PCR demonstrates autosomal trisomy in 30%, karyotyping detects pathogenic unbalanced chromosomal rearrangements in 5%, and CMA detects imbalances in up to 6.5% of structural abnormal fetuses.[3,4] Therefore, approximately 60% of these pregnancies do not have a prospectively identified genomic diagnosis that would guide prognostic information giving and counselling.[5] A proportion of these 'unsolved' cases may be the result of monogenic disease. Information from a family pedigree, previous obstetric history and knowledge of consanguinity may be helpful in the assessment of these pregnancies. Specifically, in fetal medicine, due to the limitations of antenatal imaging, there is an inability to identify subtle dysmorphic features, which hinders the ability to narrow the differential diagnosis. In addition, fetal structural anomalies often vary in expression at ultrasound detection at different gestational ages. These factors limit complete phenotypic information especially in the second trimester, when the initial concern about fetal anomaly typically occurs.

Next-generation sequencing (NGS), also known as high-throughput sequencing, is the catch-all term used to describe a number of different modern sequencing

technologies. Whole genome sequencing (WGS) and exome sequencing (ES) provides high resolution testing of DNA down to the single base-pair level. For WGS, both the non-coding (introns) and coding (exons) regions on DNA are sequenced without prior selection. For ES, DNA regions of the 'protein-encoding' exons which make up 1–2% of the genome containing >85% of all disease-causing pathological variants, are captured prior to sequencing.

In WGS, each of the 3 billion bases is sequenced multiple times. Genomic DNA (gDNA) is initially fragmented into a library of small segments that are uniformly and accurately sequenced in parallel reactions. The newly identified strings of bases, called 'reads', are then reassembled using bioinformatic software using a known reference genome as a scaffold (a process called alignment). Multiplexing enables large sample numbers to be simultaneously sequenced in a single experiment. In the process, individual 'barcode' sequences are introduced to each sample so they can be differentiated during the data analysis.

An alternative to WGS is to sequence only the exomic regions (ES) or part of the exome in a 'panel' of (pre)selected genes/variants (see text). To achieve this, a 'target capture' step is introduced at the start of the process to select out from the gDNA only those genes or exomic regions to be sequenced.[2]

Information from cohort studies using prenatal WGS is uncommon but is increasing in number. At present relating to the use of prenatal WGS, the literature consists of a single case study identifying disruption of the CHD7 gene in a pregnancy complicated by multiple anomalies (micrognathia, arthrogryposis and polyhydramnios).[6] In addition, two retrospective cohort series of fetuses with anomalies have been described: one focusing upon increased nuchal translucency in the first trimester[7] and the other a mixture of prenatal and postnatal cases investigated using 'low pass' WGS.[8] Theoretically, such testing will also detect fetal aneuploidy and copy number variation (which may ultimately bypass the need for prior karyotyping and chromosomal array testing), but bioinformatic filtering and interpretation are more complex, and the methodology is at present more expensive. However, it is likely that in the future, more data will become available on the use of prenatal WGS and this technique has been favoured by the 100,000 Genome Project in the UK. Because of limited data for prenatal WGS, this review will primarily focus upon prenatal diagnosis using ES in the evaluation of fetal structural abnormalities and the expanding evidence base for its utilisation.

This article focuses upon the evidence for prenatal exome sequencing in the prospective evaluation of fetuses with ultrasound identified structural anomalies. It describes the clinical infrastructure required to be in place to optimise pre-test parental counselling, fetal case selection, laboratory test selection (including bioinformatics pathways and variant calling), the use of exome panels restricted to causative (postnatally defined) known genetic syndromes, the clinical review of 'potential variants' and potential ethical dilemmas that may ensue – all this delivered in a rapid turnaround time (TAT) consistent with those adopted for rapid emergency genetic diagnoses in neonatal and paediatric intensive care settings.[9]

Existing prospective, prenatal exome sequencing studies

In the field of paediatrics, two major prospective cohort studies comprising heterogeneous phenotypes (many associated with childhood neurological sequelae and developmental delay) have demonstrated a diagnostic 'pathological variant' yield in up to 30% of cases, aiding understanding and management and allowing discussion of recurrence.[10,11] In contrast, in obstetrics the majority of studies reported since 2010 describing the use of prenatal exome sequencing in the evaluation of fetal anomalies are small, retrospective single or cohort case series. An informative systematic review including data from peer-reviewed published papers, published retrospective cohort series and published conference abstracts (containing at least five cases) has been published by a multi-institutional group of experts.[12] That publication collated data from 16 'citations' which contained a range of inclusion criteria: fetuses with isolated structural abnormalities, fetuses with multiple fetal abnormalities and those with an increased first trimester nuchal translucency (NT). In addition, the studies included different proportions of fetal anomalies associated with miscarriage, perinatal mortality and termination of pregnancy. Many reported fetus-only ES testing, whereas others included 'trio testing' (fetus, mother and father), a method proven to increase the diagnostic rate.[13] These 16 studies reported a diagnostic rate that ranged from 6.2[14] to 80%,[15] across the studies[6,13–28] and documented how inclusion criteria altered the range of positive diagnoses.

However, in early 2019, two relatively large, prospective-recruited cohort studies (from the UK and USA), targeting ES in fetuses with unselected ultrasound-detected structural anomalies, were published in *Lancet*.[29,30] A total of 844 fetal probands, eligible consented 'fetal trios', where QF PCR and CMA had previously been performed and was
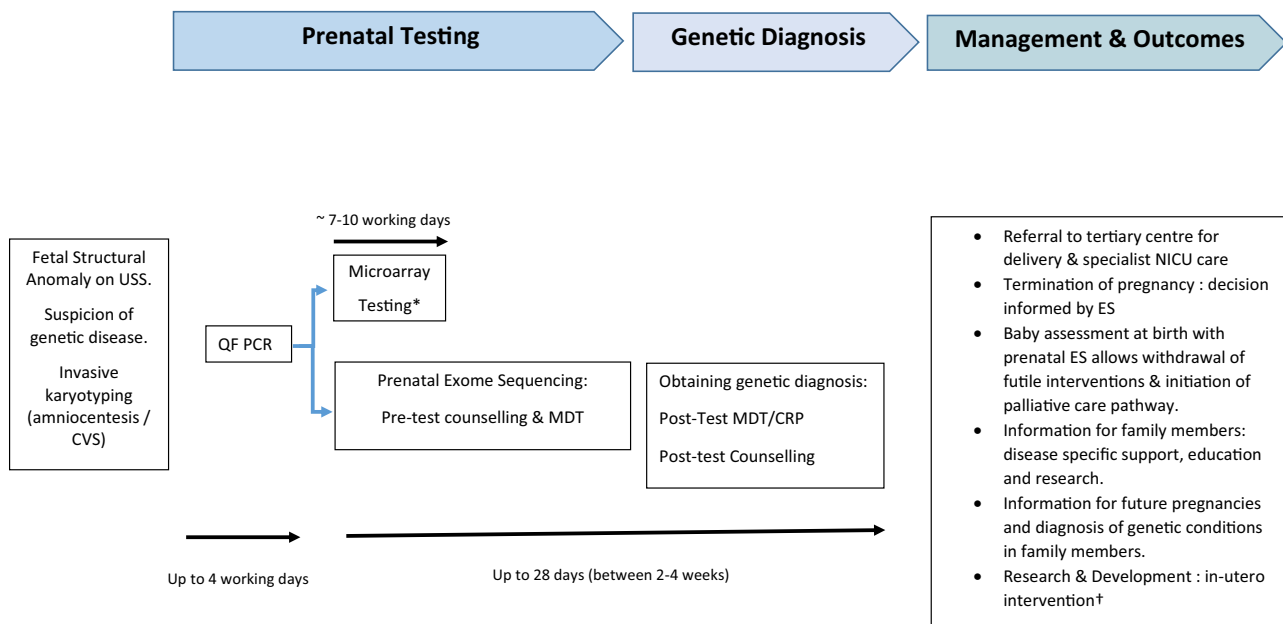
Figure 1. A schematic diagram of the integration of prenatal ES into the current prenatal antenatal diagnostic pathway. Prenatal sonogram (USS), quantitative fluorescence-polymerase chain reaction (QF PCR) (to exclude autosomic trisomies & monosomy X). MDT, multidisciplinary team meeting; CRP, clinical review panel. *Microarray testing would be initiated in tandem with prenatal ES. †Such as prenatal mesenchymal stem cell transplants by in utero transfusion in OI (BOOST4 study).[65]

normal, were studied (Figure 1). In 76 probands (9%), a causative pathological genetic variant was identified.

The largest of these was the Prenatal Assessment of Genomes and Exomes (PAGE) study, which performed ES on 610 trios in cases of an identified fetal anomaly and where CMA was negative. Overall, ES provided an additional 8.5% diagnostic yield of pathogenic variants compared with conventional genetic testing. In addition to the pathogenic variants considered to be a direct cause of the relevant fetal structural anomaly, variants of uncertain significance (VUS) and of potential clinical relevance were diagnosed in a further 4% of cases.[29] The second prospective prenatal series conducted by Columbia University reported an additional diagnostic yield of 10.3% (in 234 trios).[30] Both these studies gave diagnostic yields that were lower than those reported from retrospective, highly selected case cohort studies.[12]

Fetal phenotype

The two prospective studies recruited pregnancies in which the fetus had an abnormality identified by ultrasound and the parents opted for antenatal karyotyping (by QF PCR/CMA). Both studies therefore were pragmatic in the identification of an unselected cohort of fetuses with structural malformations in a routine fetal medicine clinical setting.[29,30] The consequence of this was a heterogeneous mix of anomalies. Both studies recruited a high proportion of

fetuses with multiple (>1) structural anomalies. The Petrovski study had a proportion of pregnancies that, although unselected, included cases of cardiac rhabdomyomas and infantile polycystic renal disease (likely to be associated with genetic disease).[30] In these latter cases, perhaps 'targeted testing' could have been utilised.

Both studies found a greater diagnostic rate of pathogenic variants in the presence of multiple congenital anomalies (between 15.4 and 18.9%, respectively).[29,30] This opens a debate as to whether specifically to select fetuses with specific single anomalies or more than two major abnormalities for testing, and whether testing should be broad or based upon a 'targeted' virtual panel dependent on the presenting phenotype. High resolution ultrasound has led to the diagnosis of structural anomalies in the fetus with variable rates of detection,[31] even with the use of additional modalities such as magnetic resonance imaging.[32] Subtle dysmorphic features may not be diagnosed and variability of phenotypic expression, incomplete penetrance and varying gestation of presentation make the identification of the fetus at risk of monogenic disorders challenging. The PAGE study identified specific phenotypes that were associated with the highest yield of pathological variants. These included multiple anomalies (15.4%), anomalies of the skeletal (15.4%), cardiac (11.1%) and spinal systems (10%) as well as the presence of non-immune fetal hydrops (9%) (Figure 2). After correction for multiple testing, the detection of pathological (or likely
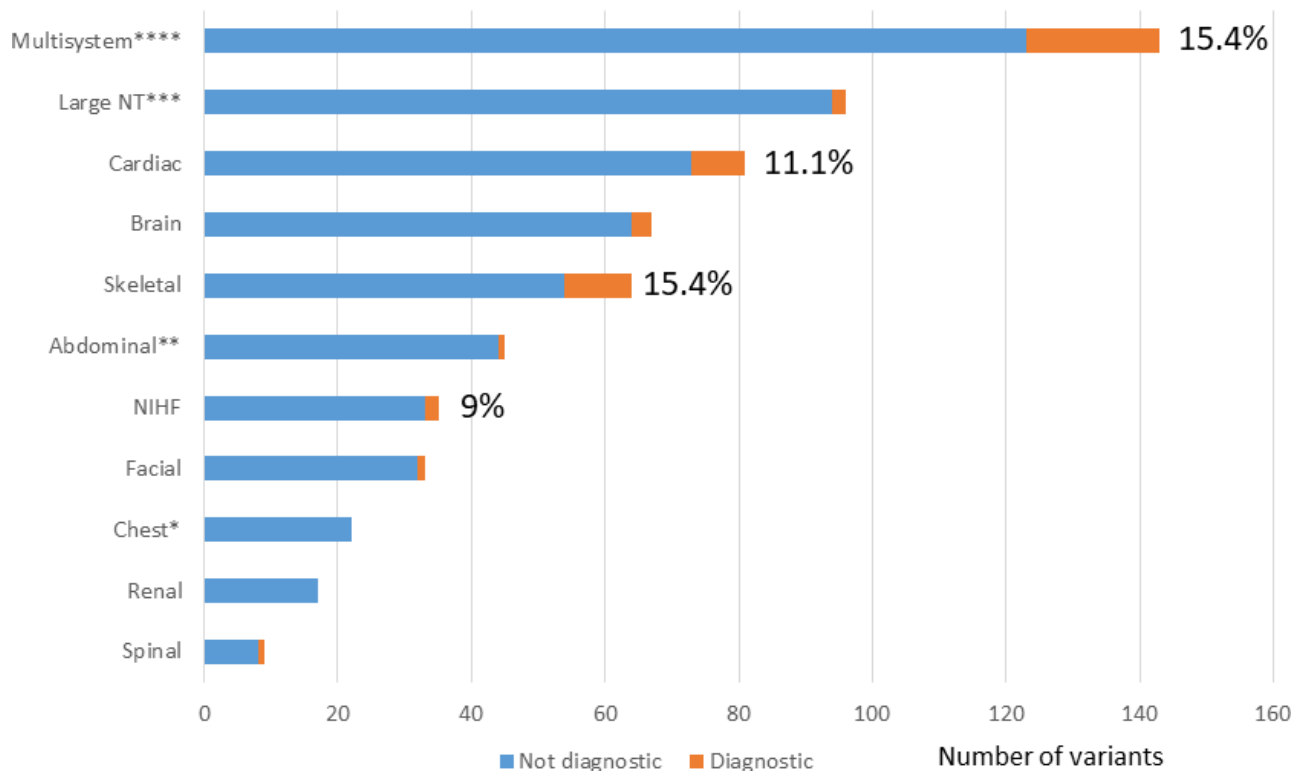
Figure 2. The proportion of diagnostic genetic variants (as a percentage of the total) identified in fetuses with each phenotypic abnormality. *Includes congenital diaphragmatic hernia. **Includes anterior abdominal wall anomalies. ***NT, nuchal translucency (>4 mm). **** Multisystem: >two structural anomalies) (After the PAGE Study).[29]

pathogenic) variants in a fetus with multisystem anomalies was significantly more likely than in a fetus with any single abnormality ($P = 0.018$).[29] The Petrovski study demonstrated a similar association with structural phenotype; however, renal anomalies (in several fetuses with infantile polycystic disease) were associated with significant monogenic disease (16%).[30] From the PAGE study, large nuchal translucencies (>4 mm) had a relatively low association with a pathological variant (on subsequent scanning at 20 weeks' gestation, additional anomalies were identified).[33] More recently published data evaluating an approach to integrating ES for fetal structural anomalies into clinical practice, also noted the highest yields of pathological variants associated with cardiac and central nervous system, renal, skeletal anomalies, as well as hydrops fetalis and arthrogryposis.[34–36] The NHS England (Genomics) are developing testing criteria based on ultrasound to guide discussions around suitability for prospective prenatal ES (Figure 2). It should also be recognised that mutations in the same gene can cause different prenatal and postnatal phenotypes and may differ with the same underlying genetic pathological variant. A good example of this is the presence of mutations in the histone methyltransferase KMT2D and the demethylase KDM6A genes[37] associated with Kabuki syndrome. In childhood, this is characterised by classic facial gestalt, multiple organ malformations, abnormal postnatal growth and intellectual disability.[38] In prenatal life, Kabuki syndrome appears to be associated with nuchal oedema, hydrops fetalis, cardiac and renal malformations, intrauterine growth restriction and associated polyhydramnios.[29,30,39] Case selection of prenatally identified abnormal fetuses, after genetics review and additional phenotypic information from autopsy, increase diagnostic yield from an index pregnancy.[35,36] Published postmortem series suggest a yield from ES of up to 30%, reflecting the importance of case selection by detailed, accurate phenotyping (with the inclusion of subtle dysmorphic features).[10,23,36,39] Obviously, paediatric follow up, and in particular the identification of neurodevelopmental and intellectual disability (not definable prenatally), may also indicate the potential underlying aetiology.[10,11]

Bioinformatics and interpretation of variants identified using ES

In the PAGE study, sequenced data were assessed for candidate pathogenic variants from a selected group of exons constituting the DDG2P list of 1628 genes, previously used in the DDD study.[11,40] These were selected as they were identified as rare, protein-altering variants in which the

inheritance pattern of the variant matched that of the gene being assessed for clinical review (by bioinformatic filtering).[29,40,41] Trio (fetal, paternal and maternal DNA) analysis was used, as it enables filtering of variants according to inheritance patterns and therefore speeds up analysis and reporting. Determining the inheritance pattern can also be helpful in variant classification as an internal validation of the result and can also give information on recurrence risk. These 'candidate pathological variants' were then fed into and reviewed by the clinical review panel (CRP), a multidisciplinary group of clinical geneticists, fetal medicine subspecialists, two clinical scientists and a genetic bioinformaticians) who reviewed anonymised variant annotation data and clinical findings using the SAPIENTIA software (version 1.75; Congenica, Cambridge, UK). The CRP reached a consensus view as to the variant classification (i.e. pathological, likely pathogenic, variant of unknown or uncertain significance), likely benign and or not benign, and the likelihood that it was the cause of the fetal phenotype. Using this methodology, an average of 0.4 variants were reviewed per proband (fetus with structural anomaly).[29]

The Petrovski paper used similar but not identical methodology for identification of pathological variants.[30] Again, they used trio analysis and a previously published framework to allow rapid and efficient identification of de novo and inherited variants.[41] This focused upon two 'tiers' of qualifying genotypes. Tier 1 was associated with the assumption that a relevant genotype would be highly penetrant and be absent from the parents (and controls). Tier 2 was a literature-motivated screen, which permitted genotypes to be observed at low frequencies among controls (internal and external) which had to have been previously classified as pathogenic on *Clinvar* or Human Gene Mutation databases.[42,43] Again, potential causative variants were classified by a multidisciplinary conference of specialists to agree genotype/phenotype causation. This methods analysed all genes and also incorporated 'bioinformatic signatures', assessing variants in genes that were not yet linked to disease. This resulted in a ten-fold increase in the variant interpretation burden compared with PAGE (4.8 variants per case versus 0.42 per case requiring manual interpretation by PAGE) (due to the study the whole exome [~20–25 000 genes] was analysed rather than a selected panel of genes [1628 genes in PAGE]), with a limited difference in the overall final pathogenic variant yield.[30] This demonstrates the need to balance a higher diagnostic yield with higher interpretational burden in a prenatal ES strategy, as well as considering the bioinformatics pipeline adopted. It is also important to realise that causative variant association with phenotype may alter with time and the 'variant' list will need to be updated with time. This means that if the fetal trio ES were to be periodically reanalysed (during childhood) every 1–2 years, additional pathological variants might be identified. This has already been recognised in the use of ES/WGS in paediatric datasets.[40]

Secondary and incidental findings

In clinical practice, variants of uncertain significance are those where pathogenicity is unclear. If fed back to patients, they may cause significant anxiety and make patient decision-making more complex (especially in the context of a fetal structural abnormality identified in a pregnancy where termination of pregnancy is an option). Parents report that such information is 'toxic' and emotional effects may last for a considerable period.[44–46] Therefore, as prenatal ES is used to evaluate congenital malformations in clinical practice, there is a need to register all genetic variants, including VUS and the fetal phenotype in an international registry with comprehensive clinical access. Secondary findings are genetic variants, unrelated to the primary presentation (of the probands) but may be reported if deemed 'medically actionable'.[12] In a paediatric setting, the American College of Medical Genetics and Genomic (ACMG) has set out guidelines stating that when offering ES, secondary findings should be reported in 59 genes (in which it is believed that there is clinical evidence that pathological variants may result in disease that may be prevented or treated).[47] However, with this guidance, there is an exclusion of prenatal ES. The extension of this process to prenatal diagnosis (and the parental samples of trios) has been debated and is controversial.[48] An example of this would be the potential ES testing of a fetus with a phenotype suggestive of Fanconi anaemia.[49] Somatic inactivation of the Fanconi anaemia/breast cancer gene (BRCA) pathway accounts for the chromosomal instability of the predisposition of some cancers (breast, bowel and ovary) in the general population.[50] However, it is recommended that if instituted, it should be at present on a case-by-case basis, and careful and detailed pre- and post-test counselling of parents is imperative. In addition, trio ES could also reveal unforeseen issues such as non-paternity or parental consanguinity, again leading to difficult counselling scenarios.[49,51,52]

Pre-test and post-test counselling

A more than superficial understanding of prenatal ES by parents and its implications for the pregnancy, themselves, their family and future pregnancies is extremely important and cannot be overstated. In the UK, prenatal test counselling in pregnancy has been traditionally the role of screening midwives or specialist genetics counsellors, and when prenatal invasive tests are contemplated it is the obstetrician who usually discusses the procedure and laboratory test with the parents. In North America, this role is

often taken by the genetics team, commonly certified specialist genetics counsellors. The pre-test counselling for potential prenatal ES, therefore, needs to be detailed and intelligible. It must be emphasised that such testing is new and the clinical utility and the detection of specific genetic diagnoses (depending upon the fetal phenotype) are emerging from research studies and will undergo further review as these tests are utilised in clinical practice. There is broad agreement that this should be led by genetic healthcare professionals. However, the challenges of delivering such information to parents (at an emotive time in their pregnancy when a fetal abnormality has been detected) with varying educational, religious and cultural backgrounds and experiences should not be understated.[12,51,52] It may also raise ethical considerations.[51–53] Our own experience, piloting prenatal ES, is to have a multidisciplinary meeting (to discuss the case study and imaging) and to be conservative about fetal phenotype selection prior to offering the testing.[54] If the multidisciplinary team (comprising the same group of specialists as used in the PAGE study)[29] decide that the pregnant woman may be offered such testing, the couple are seen in a multidisciplinary combined fetal medicine/genetic clinic where a repeat ultrasound examination is performed (and previous information from the family pedigree, diagnostic imaging and tests reviewed). The couple then have pre-test counselling by a fetal medicine subspecialist and a clinical geneticist (Figure 1).[12,54] I have discussed this clinical pathway and the published data supporting its use in the accompanying presentation given in September 2020 (Video S1).

Once the laboratory-based ES and potential variants have undergone bio-informative filtering, a multidisciplinary clinical review panel is essential to discuss the clinical significance of any variant identification and to elucidate the potential likelihood of causation in the associated fetal abnormality or abnormalities. Once a causative genetic variant has been identified, this again needs to be discussed with the parents in a multidisciplinary clinic with the clinical genetic team explaining clinical relevance, potential inheritance and any further testing (especially of family members). In addition, it is not currently possible for either WGS or ES to accurately read every single base pair of every gene and therefore identify every genetic variant. Also, as experience groups and variant matching to phenotype (and pregnancy outcome experience) grows, it is likely that non-informative variants will be reclassified as pathological in time.[55] Such complex clinical pathways and infrastructure are starting to be developed in the UK, and multidisciplinary research (through the Optimising EXome PREnatal Sequencing Services [EXPRESS study]) is underway to aid the development of such pathways and to ensure equity of access and high clinical standards across the UK.[56] However, internationally, different healthcare systems have different methods of delivery of this service.

Clinical pathway 'turnaround time'

In the PAGE study, an a priori protocol decision was made only to feedback information on pathological variants at the end of the pregnancy.[29] The Petroski study (which considered that the results of ES were not intended for use in clinical care) described that it took up to 8 weeks to obtain and interpret results.[30] Data from paediatric cohorts of critically ill neonates and infants have indicated that rapid ES testing and evaluation is possible (sometimes within 72 hours) and provides clinical benefit and improved decision-making, aiding developmental and family emotional outcomes.[9,53,57–59] In prenatal diagnosis, there is also a requirement for a relatively rapid TAT to aid informed parental (in terms of consideration of termination of pregnancy) and clinical (in terms of evaluation of active versus palliative newborn care) decision-making. TAT is affected and influenced by the whole pathway (including the potential need for cell culture to obtain fetal DNA, through the genetic variant bioinformatic filtering and clinical review panel assessment). Further time savings may be made by initiating ES once the results of QF PCR are known and in parallel with CMA analysis (Figure 1). Prenatal TAT therefore can be prioritised when there is a need to obtain a result by a specific gestation time and some centres have achieved this within an average of 14 days (range 7–38 days).[34–36] A recent small retrospective study from the Netherlands indicated that prenatal ES aided parental decision-making (including decisions on late termination of pregnancy) and aided prenatal/neonatal clinical pathways. Again, on average, TAT was possible within 21 days.[60] Certainly, parents (pre-testing) must be aware that test TAT can be of variable duration, with communication between the clinicians and scientists within the laboratory being crucial.

Cost effectiveness

This will be affected by selection of phenotype, technical considerations such as DNA sequencing, variant interpretation, as well as the infrastructure for pre- and post-test counselling.[12] The use of ES in suspected monogenic disorders (in children) has been indicated to be increasingly cost-effective as the benefits of ES data reanalysis, cascade testing in first-degree relatives, and parental reproductive outcomes are incorporated into modelling.[61] Our own, rather conservative cost modelling used a decision tree model populated using data from a prospective cohort of women undergoing invasive diagnostic testing. A comparison of four potential testing strategies (after screening for autosomal trisomies) were evaluated using CMA, ES, CMA followed by ES ('stepwise'), and CMA and ES combined.

Consideration should be given for ES testing if a fetus with multiple multisystem major structural and selected other abnormalities are detected on fetal imaging. It is essential that multidisciplinary review to include clinical genetics, tertiary fetal medicine specialists, clinical scientists and, where appropriate, relevant paediatric specialists considers if a monogenic malformation disorder is likely and that the molecular diagnosis may influence pregnancy or early neonatal management in the index pregnancy.

Clinical examples include of fetal 'phenotype' include:

1. Fetuses with multiple anomalies.

2 Suspected skeletal dysplasias (IUGR should be excluded).

3. A fetus with large echogenic kidneys with a normal bladder.

4. Major CNS abnormalities (excluding neural tube defects). Mild ventriculomegaly should only be considered as an abnormality if the posterior horn is persistently >11mm. Under these circumstances it is not considered a major CNS abnormality in isolation.

5. Multiple contractures (excluding isolated bilateral talipes).

6. Persistent nuchal translucency (>3.5mm) can **only** be considered in the presence of other structural abnormalities in two or more systems.

(Minor 'markers of aneuploidy' – choroid plexus cysts, echogenic foci, mild renal pelvis dilation, small nasal bone, long bones on 3rd centile etc are **excluded**).

Figure 3. Fetal phenotypes for consideration of prenatal exome sequencing (Rapid Exome Sequencing Service for fetal anomalies testing, NHS England and NHS Improvement, 2020).

When ES was priced at GBP 2,100 (EUR 2,407/USD 2,694), performing ES alone prenatally would cost a further GBP 31,410 (EUR 36,001/USD 40,289) per additional genetic diagnosis, whereas stepwise ES would cost a further GBP 24,657 (EUR 28,261/USD 31,627) per additional genetic diagnosis. When ES is priced at GBP 966 (EUR 1,107/USD 1,239), performing ES alone prenatally would cost a further GBP 11,532 (EUR 13,217/USD 14,792) per additional genetic diagnosis, whereas stepwise ES would cost a further GBP 11,639 (EUR 13,340/USD 14,929) per additional genetic diagnosis. The sub-group analysis suggests that performing stepwise ES on cases indicative of multiple anomalies at ultrasound scan versus cases indicative of a single anomaly, is more cost-effective compared with using ES alone.[62] It is likely that these are conservative costs and different healthcare economies will need to evaluate their costs and make decisions as to whether it is possible to implement prenatal exome sequencing within their healthcare systems. Furthermore, this health economic analysis did not take into consideration, detailed and varying neonatal and long-term paediatric care costs, which are an important consideration.

The potential implementation in the prenatal diagnostic pathway in England

In England, a national NHS Genomic Medicine Service infrastructure based around seven laboratory hubs across England is in development. It is envisaged that prenatal case selection will be by a multidisciplinary (tertiary) team, led by a clinic (1) multiple anomalies and structural anomalies where there is a strong suspicion of genetic aetiology (Figure 3). The laboratories charged with providing prenatal ES would use a modified panel of pathological variants identified in the PAGE study (DD-G2P/PAGE), using a traffic-light rating and comprising almost 1000 genes that have been chosen after expert review and agreed by NHS Genomic Medicine Service sign off (https://panelapp.genomicsengland.co.uk/panels/478/).[63] As indicated in the above discussion in this review, the clinical infrastructure for delivery of this prenatal service needs to be conservative but robust. It is, however, also possible that 'pattern recognition' of fetal anomalies may lead the MDT to consider panel testing. Such panel tests are specifically designed to interrogate a set of disease-associated genes. The desirable clinical and laboratory infrastructure required will be informed by prospective, NIHR-funded research through the EXPRESS study.[56] However, it is probable that phenotypic inclusion, the pathological variant list and the clinical infrastructure for delivery will remain under continuous review and there is a recognised need for the formal curation of pathological, probable pathological and VUS variants matched to detailed phenotyping within a database such as ClinVar.[42,64] It is also likely that a strategy for re-testing will be required, as this has been noted to be important in paediatric testing strategies.[55]

Conclusion

The implementation of prenatal ES into clinical practice to evaluate the fetus with structural anomalies provides an exciting opportunity to improve delineation of prognosis, provide clinical utility and to understand further the pathogenesis of prenatal genetic disorders. It is also possible that, going forward, the identification of prenatal pathological

variants associated with structural anomalies (i.e. severe skeletal dysplasia associated with osteogenesis imperfecta) may provide opportunities for antenatal therapy (such as mesenchymal stem cell transplantation); as in the pan-EU Brittle Bone Before Birth (BOOSTB4) study.[65] However, as well as a robust, relatively conservative infrastructure for clinical delivery of this service, there is a need to continue to debate the societal, moral and ethical issues surrounding the implementation of such science so as to provide additional prognostic information to parents while attempting to limit unnecessary emotional burden on parents and society.[51–66]

Disclosure of interests

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Contribution to authorship

MDK had the idea for this review and planned, researched, wrote and revised this article.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Video S1. PowerPoint and video presentation. ∎

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