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Abstract

Introduction:

Prenatal exome sequencing (ES) allows parents the opportunity to obtain a rapid molecular diagnosis of monogenic etiology when their fetus is found to have structural anomalies detected on prenatal ultrasound. Such information can improve antenatal and neonatal counselling, decision making and management, and expand reproductive options in subsequent pregnancies.

Areas covered:

This review appraises the evidence, from a comprehensive search of bibliographic databases, for the introduction of ES into the fetal medicine care pathway when investigating congenital malformations. The perspectives of clinical geneticists, clinical scientists, fetal medicine specialists and patients are explored in relation to the novel investigation and the benefits and challenges of its use in ongoing pregnancies with particular reference to UK medical practice.

Expert opinion:

ES provides a genetic diagnosis for more than 1 in 10 fetuses with structural differences on ultrasound and normal conventional tests (karyotype or chromosomal microarray) in carefully selected cases. The diagnostic rate increases for certain phenotypes and can range between 6 and 80% where conventional cytogenetics have not detected a diagnosis. Expert oversight is required to ensure that patients receive high quality, evidence-based care and accurate counselling, supported by a multidisciplinary team familiar with the test and its implications.

Keywords

Exome; fetus; genome; sequencing

Article highlights

- Prenatal exome sequencing may identify a genetic cause for fetal malformations where traditional cytogenetic testing has not.
- The clinical application of a prenatal exome requires multidisciplinary team of experts to assess phenotypes, provide pre-test counselling and to interpret the relevance of pathologic variants.
- There may be unexpected findings which require careful interpretation and communication.

1. Introduction

Whole Genome Sequencing and particularly prenatal exome sequencing (ES) provide an opportunity to interrogate the fetal genetic code to a greater extent than previously possible. Whilst this advance in genomic technology has the ability to enhance perinatal care, it also brings new challenges. In this review we explore the evidence base for prenatal ES. Our literature search included MEDLINE, EMBASE and CINAHL from inception to January 2021, as well as relevant reference lists. Important considerations for stakeholders, including parents and clinicians, who must critically appraise the clinical utility of this rapidly evolving tool, are outlined. The multi-step scientific approach to the test, from identifying a case in which its application may be beneficial, to obtaining and communicating a result and its implications (which involves many highly trained professionals) is described in detail.

2. Prenatal genetics

Prenatal ultrasound screening may detect fetal structural anomalies (the majority being congenital malformations) in up to 5% of pregnancies and is well established in routine clinical obstetric pathways in the United Kingdom [1,2]. Congenital malformations are associated with miscarriage, stillbirth, neonatal death and long-term pediatric morbidity [3,4]. The prognosis will depend on the type of malformation identified, whether it is isolated or associated with additional structural abnormalities and any identifiable underlying etiology (chromosomal or single gene anomaly, or an etiology such as viral infection) of the structural differences. If an underlying genetic diagnosis is suspected and subsequently identified, this information will improve counselling, aid decision-making and inform clinical care (both prenatally and into childhood) [5,6].

Established diagnostic tests in routine use for prenatal diagnosis of chromosomal aneuploidy, structural variants and copy number variants (CNVs) include amniocentesis, placental (chorionic villi) biopsy and more infrequently, in modern practice, fetal blood sampling. Although cell-free fetal DNA-based non-invasive prenatal testing (NIPT) of maternal blood can provide sensitive and specific screening for specific trisomies [7], confirmation of fetal aneuploidy requires invasive testing and detection with quantitative

fluorescence-polymerase chain reaction (QF-PCR), especially in the presence of a fetal structural malformation. Conventional G-band karyotyping provides nominal and positional information to a microscopic level (resolution to 5–10Mb) [8], and chromosomal microarray analysis (CMA) further may detect copy number variation (chromosomal deletions, duplications and unbalanced rearrangements) to the level of ~1kb (dependent on platform used) [8]. Overall, stepwise, conventional invasive testing and QF-PCR/CMA testing reveals autosomal trisomy in 30%, unbalanced chromosomal translocations in a further 5% and pathogenic or likely pathogenic CNVs in an additional 4–10% of fetuses with antenatally detected structural anomalies [1,8].

A proportion of the remaining 60% of pregnancies with fetal structural anomalies are due to disease of monogenic etiology, caused by variation down to the level of a single base pair, requiring interrogation with modern molecular techniques. However, delineating genotype-phenotype correlations is challenging as fetal presentations are heterogeneous, non-specific and develop with advancing gestational age. Moreover, some phenotypes are not yet clearly defined. A given fetal phenotype may be associated with a large number of candidate genes, and some of the genes are pleiotropic. Sequentially analyzing a number of candidate single genes with traditional molecular techniques such as Sanger sequencing is cumulatively expensive and incompatible with the rapid turnaround time (TAT) required to inform antenatal care. Prior to next generation sequencing technologies, molecular testing was restricted to highly specific presentations or known family variants.

2.1 Next generation sequencing

Next generation sequencing (NGS) technology has enabled the simultaneous molecular analysis of a large number of genes at a comparatively low cost [9]. This has prompted an “explosion” in gene discovery research and subsequent applications in clinical care, mostly in rare disease and cancer [10-12]. An NGS test is a complex multi-step semi-automated process requiring input from clinicians, scientists and bioinformaticians. The key stages are DNA sequencing and data analysis, which requires identification, filtering and interpretation of variants. The methodological approach to each step varies considerably depending on preferred laboratory techniques and the aim of testing.

NGS refers to a diverse group of high-throughput sequencing technologies developed over the last decade [9], although they share the same basic steps. It can be used to sequence single genes, gene panels (multiple genes), exomes or whole genomes [9]. After basic

checks to confirm identity and rule out contamination, genomic DNA (gDNA) is fragmented into a library. Regions of interest (e.g. a gene panel or clinical exome) are captured and amplified in an optional target enrichment step. Massively parallel sequencing detects and produces millions of reads. Bioinformatics software aligns the reads to a reference genome and carries out quality control checks. One of the most common NGS technologies currently used in medical genetics is the ‘sequencing-by-synthesis’ technique [9], and this technology has largely been integrated into National Health Service (NHS) laboratories (in the United Kingdom) to embed genome sequencing technologies in mainstream clinical services. DNA samples are pooled in one sequencing reaction by attaching a molecular barcode unique to each sample to every DNA fragment, increasing throughput and cost effectiveness [10].

In the analysis stage, the differences between the reference sequence and the test sequence are identified, assessed for pathogenicity, filtered and prioritized using complex automated pipelines curated by bioinformaticians following best practice consensus guidelines [13]. Analysis is often targeted and restricted to clinically relevant genes by applying virtual gene panels. In variant calling, valid differences between the test sequence and the reference genome are identified. The variants are annotated with contextual information including gene position, allele frequency, inheritance pattern, known and predicted pathogenicity data and predicted phenotype. This is achieved with comparisons to disease and population databases (for example gnomAD [14] and ClinVar [15]), in silico prediction tools, published literature and using standardized descriptors such as The Human Phenotype Ontology (HPO) terms [16]. Annotated data is filtered and prioritized to highlight clinically relevant variants (rare, pathogenic variants associated with the observed phenotype and inheritance pattern), which are presented to clinical scientists for detailed appraisal of pathogenicity using standardized consensus guidelines [17].

NGS usually entails either whole genome sequencing (WGS), which examines the entire genome, or ES, which examines only the ‘protein-encoding’ exons. The exons make up to 2% of the genome but contain more than 85% of all disease-causing pathological variants. ES is more commonly used in current prenatal diagnostic testing [5,18]. It provides a compromise between gene discovery potential and the practical issues of whole genome sequencing (WGS), which is more computationally complex, expensive and requires more DNA [19,20]. ES can detect single nucleotide variants (SNVs), small insertions and deletions (indels), and some copy number variants (CNV) [13]. Results must always be reviewed in the context of the fetal ultrasound scan findings and other relevant information, such as family

history (pedigree), and additional specific genetic or biochemical tests or further imaging may be considered.

Prenatal WGS cohort studies are limited and generally focused on detection of CNVs and comparison with CMA. For example, a retrospective cohort study reported diagnosis in 16 of 50 fetuses (32%) with structural anomalies caused by a mixture of CNVs and single nucleotide variants (SNVs) [21]. Retrospective cohort studies have also evaluated low-pass WGS for detection of clinically significant CNVs including in the prenatal cohort [22-24], and a published case report detected a pathogenic CHD7 variant in a fetus with hydrops using whole-genome 'jumping libraries' methodology [25]. In time, WGS may supersede CMA and ES as a more effective and comprehensive single-test method to detect CNVs and SNVs, including small CNVs not detectable with CMA or ES. Currently, barriers of cost and technology prevent this.

2.2 Prenatal exome testing

A 2017 systematic review of prenatal genomic sequencing studies and conference abstracts [26] reported a significant wide range of diagnostic rates of between 6% and 80% in retrospective case cohort studies [27,28]. Of 31 studies identified, 16 reported cohorts of 5 probands or greater. Most of these studies were enriched retrospective case series or case reports. Sample size, filtering methodology (either fetus alone or trio comprising the fetus and both parents) and cohort selection based on fetal phenotype was likely to account for much of the variability [26].

The largest two cohort studies prospectively recruited a total of 844 pregnancies with fetuses with at least one major structural difference from Fetal Medicine Centers and notably revealed a more conservative combined diagnosis rate of 9%. The structural anomalies were selected not based upon the potential risk of monogenic disease but, a priori, based upon a clinical indication to karyotype the fetus and therefore exclude single gene anomalies (if karyotype was normal). Both studies used trio WES (fetal, maternal and paternal DNA analysis) in fetuses with abnormal ultrasound and normal QF-PCR and CMA. The multicenter UK-based Prenatal Assessment of Genomes and Exomes (PAGE) study of 610 trios revealed a diagnosis in 8.5% and a potentially clinically useful variant of uncertain significance (VUS) in 3.9% [18]. In a prospective cohort study from the USA, Petrovksi and colleagues reported a diagnostic yield of 10% in 234 trios, with variants with 'bioinformatic signatures indicative of pathogenicity' in a further 20% [5]. Both studies highlighted the utility

and feasibility of introducing prenatal ES in routine Fetal Medicine care as well as the limitations, potential difficulties, and need for robust care pathways with specialist multidisciplinary team (MDT) working. Subsequently published studies, a mixture of phenotypes in prospective and retrospective cohorts, revealed diagnostic yields of 20–35%[29-38], while an enriched cohort of 50 lethal autosomal recessive disorders (n=50) yielded a diagnosis in 52% [39].

Prenatal ES is increasingly being incorporated into routine genetic services, including into NHS-funded care in the England in October 2020 [40]. The Optimising Exome PREnatal Sequencing Services (EXPRESS) study, a formative and mixed-methods evaluation of the NHS service, is currently underway and aims to evaluate ethical, financial, clinical and logistical issues to inform optimal service delivery [41]. Workstreams include research into both staff and patient experience, to find out the successes and challenges of the current service.

3. Clinical Scientist perspective

3.1 Clinical pathway

A rapid turnaround time is required for ES to inform the care of an ongoing pregnancy. This was not included in the study design in the two 2019 prospective fetal ES studies, with results taking up to 8 weeks to report in the Petrovski study [5]. However, multiple laboratories have demonstrated an average TAT of 14 days (range 7–38 days) from DNA extraction to result [29,34,42]. This requires streamlining of laboratory workflows, which includes sample receipt, culture, DNA extraction, identity checks, library preparation, sequencing, bioinformatic processing, variant filtering and assessment, and reporting [13]. The recently launched NHS service aims to issue a provisional report in 14 days, with the finalized report and confirmation of results by 21 days [43].

3.2 Bioinformatics and variant filtering

A range of bioinformatics strategies is seen in the published literature, reflecting a difficult balance between gene discovery and minimizing interpretational burden. Trio analysis is associated with increased efficiency in assessment compared to fetus alone, as the inheritance of variants can be filtered and assessed rapidly [18,30,44]. In the PAGE study,

bioinformatics analysis of sequenced exome data was restricted to a virtual gene panel of 1628 clinically relevant genes curated in a landmark ES study in rare disease [11,18]. Rare, protein altering variants with a consistent inheritance pattern were prioritized for anonymized review by a multidisciplinary clinical review panel [18]. This strategy identified 0.4 variants for clinical review *per proband* [18].

Petrovski and colleagues applied a 'gene-naïve' approach to analysis. Variants were reported in two tiers. Tier 1 variants were de novo, rare and highly penetrant. Tier 2 variants had lower variant calling requirements and included variants classified as pathogenic by ClinVar [15] or the Human Gene Mutation Database [45], or protein-truncating variants affecting a known-disease causing gene [5]. The variant-interpretation burden for the multidisciplinary panel was higher at *4.8 variants per proband* but resulted in minimal difference in final diagnostic yield (8.5% vs 10%).

The authors of the PAGE study advocated for ongoing curation of detailed genotype-phenotype gene lists, which could allow smaller, phenotype-specific gene panels to be applied to reduce variant interpretation load and incidence of VUS and secondary findings [18]. This approach was subsequently used in a cohort of 16 fetuses with skeletal dysplasia, where virtual panel of 240 genes resulted in a diagnosis in 80% [42]. The new NHS test uses a bespoke gene panel (~1000 genes) curated on the open-source online platform PanelApp, which was approved by the Genomic Medicine Service in August 2020 [46]. This is under constant review but keeping pace with new genes is challenging.

Actively refining bioinformatics pipelines is beneficial. For example, two inherited cases of Noonan syndrome were initially filtered out in the PAGE study because the parents were thought to be unaffected. The bioinformatics pipeline was adapted to include reviewable 'whitelist' of important pathological variants inherited from a parent to ensure variants were not missed and to prompt targeted and detailed assessment of parental phenotype [18].

3.3 Variant interpretation and variants of uncertain significance

Variant interpretation involves rigorous appraisal of multiple sources of evidence and is subject to individual and lab-specific variability [13]. Variants are diagnostic and reported to families if they are pathogenic/likely pathogenic and causative/likely causative of the phenotype [5,18]. In published studies and in clinical practice, pathogenicity status is reached

by consensus of an expert multidisciplinary clinical review panel following American College of Medical Genetics and Genomics best practice guidelines [17,47].

Phenotypic data, expressed via HPO terms or other standardized terminology, enables the effective analysis and prioritization of variants using an application called Exomiser [48]. However, there is a paucity of published evidence regarding the prenatal phenotype of known monogenic disorders, making it sometimes challenging to confidently assign causality to a variant. Evaluation of phenotype is also challenging due to heterogenous and evolving presentations and limitations of ultrasound compared to postnatal investigations. However, this knowledge base is expanding with increased uptake of testing, with novel presentations described in many cohort studies [29,34]. For example, the prenatal phenotype of Kabuki syndrome has been expanded to include nuchal edema, hydrops fetalis, cardiac and renal malformations, fetal growth restriction and associated polyhydramnios [5,18,36]. To keep pace with the rate of change an international, open access, anonymous detailed genotype-phenotype database is required [18], such as DECIPHER which has become widely used in rare disease [49,50]. As the knowledge base expands and gene panels adapted accordingly, a mechanism is required to reanalyze historical results that may reveal new diagnostic information, as recognized in pediatric datasets [51,52]. It is important to emphasize to clinicians and patients that exome results are not definitive and may change with time.

Cautious assessment and reporting of *variants of unknown significance* (VUS) risks providing ‘false negative’ results, which can be particularly difficult if a potentially serious condition is possible in a fetus with a relatively minor phenotype [18,53]. There are also harms associated with reporting VUS [54]. In a qualitative review of patient experience of the PAGE study, patients reported that this uncertain information was ‘toxic’ and increased the complexity of decision making [53]. In a USA-based study, ‘inconclusive-possible’ variants, found in 10 of 102 fetuses (9.8%), were fed back to families. The authors discussed the difficulty of using this information for reproductive planning options, highlighting that many Assisted Reproduction clinicians discourage selection by VUS and that highly tailored genetic counselling is required. The NHS service has opted not to include VUS in patient reports. However, there is the option of reanalyzing and assessing these data postnatally in context with more phenotypic and follow-up information and less time-pressure. An advantage of ES over WGS is that the most appropriate genes are tested, which minimises the incidence of VUS and incidental findings.

3.4 Secondary findings

Screening for pathogenic variants in unrelated 'medically actionable' genes is controversial, especially in the prenatal setting [17,55,56]. The American College of Medical Genetics and Genomics guidelines, which exclude general and prenatal populations, recommend the routine screening of 59 genes [17,57] but other institutions take a more conservative approach [58,59]. Most prenatal ES cohort studies did not intentionally screen for secondary findings [5,18,29] although in one study it was a compulsory part of the study design and medically actionable reports were issued to 2.9% (6/204) of parents [34]. In the UK, the prenatal exome panel includes some cancer genes which cause prenatal phenotypes in the biallelic form. For example, PALB2 and BRCA2, which cause Fanconi anemia in the biallelic state, but are cancer predisposition genes in the heterozygous state. Therefore, parent 'carriers' have a significant predisposition to cancer, and this will be disclosed (see discussion below).

4. Clinical Geneticist perspective

In addition to the considerations above, careful consideration of the family history (specifically if the parents have subtle features of a variable disorder such as Noonan syndrome) identification of appropriate phenotypes and pre- and post-test counselling are crucial aspects of streamlined multidisciplinary care. In cases where recurrence risks are high, planning future pregnancies and discussing the options is important. Genetic counsellors are skilled and knowledgeable communicators who play an integral role in supporting families with decision making and coordinating care delivery.

4.1 Case selection

Cohorts with the highest diagnostic yields are not necessarily the patients for which fetal ES adds the most value. Diagnostic yields are higher in cohorts with a more severe phenotype [26,33,60,61], consanguinity and family history of fetal structural anomaly [27,39]. One cohort study (n=41) reported a significantly higher yield in fetuses with abnormal ultrasound and positive family history (55.6%) compared to abnormal ultrasound alone (13%). However, ES arguably has superior utility in milder phenotypes [62] where there is a higher likelihood of providing valuable information not obtainable with ultrasound. In the PAGE study, learning disabilities were a feature of 16 molecular diagnoses made in fetuses with no CNS abnormalities on ultrasound scan. This included a pathogenic *ANKRD11* variant (associated with KBG syndrome postnatal phenotype), diagnosed in a fetus presenting with an

atrioventricular canal defect (AVSD) [18]. By contrast, 20/52 (38.5%) of diagnoses in the PAGE study were made in pregnancies ending in termination based on the ultrasound scan alone. Ongoing evaluation of patient outcome is important to ensure utility and that alternative methods of ES (e.g. postmortem or postnatal) are not more appropriate.

There are limited data about diagnostic yield by phenotype. Published phenotype categories are broad and contain significant variability (Table 1). The PAGE study was notable for including milder phenotypes such as isolated talipes and nuchal translucency, which were found to have a very low yield [18]. Interestingly, the yield for cardiac conditions was relatively high in PAGE study (11.1%) but low in the Petrovski study (5%), possibly due to low proportion of fetuses with additional extracardiac features [5]. In a recent systematic review including 636 cases of congenital heart disease, the yield for ES was 21% overall, increasing to 37% for the subgroup with extracardiac anomalies [63]. Yields are also observed to be higher in cohorts referred from clinical genetics (e.g. 88% vs 15.1% in cohorts with skeletal dysplasia and associated features, which is the highest yield group). Recognizing likely monogenic disorders is evidentially a nuanced process that requires knowledge of detailed phenotype-genotype correlations. For example, VACTERL association is a pattern of multiple severe anomalies that could appear monogenic but is usually a sporadic mesodermal defect. This again emphasizes the importance of pre- and post-test MDT working as well as sharing genotype-phenotype information to inform case selection, gene panel curation, variant interpretation and post-test counselling.

In the NHS (within England presently), clinical inclusion criteria for prenatal ES comprise major anomalies in multiple systems with a likely monogenic etiology, suspected skeletal dysplasia, large echogenic kidneys and a normal bladder, major CNS abnormalities (excluding neural tube defects), and multiple contractures (excluding isolated talipes), a nuchal translucency of > 6.5mm plus another anomaly (either in the first trimester or subsequently discovered on a later ultrasound scan), with a normal CMA and isolated second trimester non-immune fetal hydrops detected at the detailed mid-trimester anomaly scan, with a normal array [43]. Patient eligibility is discussed in a tertiary fetal genetics MDT, comprising genetics and fetal medicine consultants, clinical scientists and genetic counsellors.

Counselling involves a complex exploration of numerous options and outcomes, requiring discussion and understanding of risk, science, medicine, and ethical issues, and is usually

carried out by consultant clinical geneticists and genetic counsellors or experienced members of the MDT. In pre-test counselling it is important to be clear about the capabilities and limitations of the test. In our West Midlands Genomic Medicine NHS service, couples eligible for ES are usually invited to a joint clinic where clinical information is reviewed and repeat imaging performed [40]. Pre-test counselling is then carried out jointly with fetal medicine and genetics teams. The findings from the ongoing EXPRESS study will help to facilitate improvements in service delivery and optimize patient care. Post-test communication can be fraught with difficulty at an already emotive time, as the impact for a baby and/or the wider family may not be clear-cut, especially if there are unexpected or uncertain findings. Although a prenatal diagnosis allows detailed parental counselling, it cannot predict the severity of the postnatal phenotype (particularly any handicap and developmental delay) and the information currently available may be limited. This is particularly challenging if parents are considering a termination of pregnancy. At an already emotive time, parents may need to digest yet more unexpected information such as the risk of associated neurodevelopmental conditions. Follow-up of couples is important to discuss recurrence risks and appropriate reproductive options. This may include early invasive prenatal testing, bespoke non-invasive prenatal diagnosis (NIPD) and pre-implantation genetic testing (PGT).

5. Fetal Medicine perspective

The onus is now on Fetal Medicine specialists (working within multidisciplinary teams) to identify fetuses with structural differences eligible for prenatal ES, recognizing that non-isolated, multi-system anomalies are more likely to have underlying genetic cause. Fetal Medicine specialists are responsible for describing the phenotype accurately, using expertise in 2D and 3D ultrasound imaging techniques (and additional magnetic resonance imaging) to provide as much detail as possible about the phenotype to the genetics team, bearing in mind that the features and dysmorphology may be subtle and vary with gestation. The quality of the information in the referral will help to triage cases appropriately to whole ES or a specific panel, and to make sure that pathogenicity of variants is ascribed with as much accuracy as possible.

Fetal DNA is obtained from chorionic villus sampling (between 11 and 13 weeks), amniocentesis (after 15 weeks) or fetal blood sampling [29] and it is important to provide a sufficient sample to the laboratory. The quantity of required DNA decreases as preparation techniques improve so the cell culture step in the laboratory could eventually be eliminated,

saving significant time [13]. However, cell culture at receipt of the initial invasive sample ensures sufficient high-quality DNA for stepwise analysis by QF-PCR, CMA and fetal ES without repeat invasive testing, and DNA extraction can be initiated as soon as ES consent is provided. Further time savings are made by running CMA and fetal ES in parallel, for the majority of indications. There is a precedent for this following delivery of rapid ES for acutely unwell children with a likely monogenic disorder into the NHS a year earlier.

As more genomic information becomes available to confirm or exclude a genetic etiology, the Fetal Medicine specialist will be able to communicate the findings with the parents in collaboration with the clinical genetics team. Bespoke plans of care for the fetus and neonate can be formulated. Whilst evidence of normal genetic tests is not a prerequisite for invasive fetal therapy, an intervention such as a pleuroamniotic shunt might be inappropriate in conditions where a fetus is predicted to have a poor neonatal outcome due to its genetic diagnosis (with long-term associations with severe handicap). ES may help to identify candidates for novel gene therapies, such as the use of mesenchymal stromal cells in the prenatal treatment of osteogenesis imperfecta in fetuses with *COL1A1* and *COL1A2* gene variants [68].

6. Parents' perspective

A thematic analysis of interview transcripts from participants in the PAGE study revealed that parents struggle with uncertainty and the latency between testing and communication of results [18,69]. In seeking information some wish to confirm normality whilst others want definitive answers. Parents valued repetition of information in different formats and the support of professionals such as specialist midwives. Participants and patient groups have identified significant challenges with the consent process in ES and CMA, highlighting and accepting that comprehensively conveying and retaining complex information in a stressful environment is virtually impossible[70]. In a focus group setting, patient representation groups emphasized the importance of training professionals from a non-genetics background to reduce variability in quality and depth of counselling[71]. It is essential that patient representation and feedback of genomic services continues and is integrated into assessment and clinical audit pathways.

7. Ethical perspective

The roll out of prenatal ES in a healthcare system brings with it ethical challenges, not least

in terms of resource allocation and equity of access according to strict eligibility criteria. An existing document from the Joint Committee on Genomics in Medicine provides transferrable recommendations for the use of CMA in pregnancy [72]. Clinicians are encouraged to consider the context in which the test was done and to report variants which would affect the care of either the fetus or family now or in future. For example, a variant associated with late-onset neurodegenerative disease, a non-actionable finding unrelated to fetal phenotype, would not be reported. Incidental findings can arise from routine analysis, for example in the PAGE study where analysis for Fanconi anemia (an autosomal recessive condition) revealed a heterozygous pathogenic variant in a gene in the Fanconi/BRCA pathway, conferring increased cancer-susceptibility to the parent [18]. This was unrelated to the fetal phenotype and therefore not reported to the parent in accordance with ethical approval for the study. In practice, cancer susceptibility genes are actionable if screening can be implemented, or risk reduction surgery offered. The future autonomy of the unborn child and their right to an open future should be considered, raising ethical discussions about the scope of parental responsibility and societal attitudes towards disability [53]. Ethical principles will also apply to termination of pregnancy decisions and the storage of data. Ethically challenging results are fed back on a case-by-case basis after multidisciplinary review [5,29] and counselling should clearly define the scope of testing and potential for unexpected or difficult results, including consanguinity and non-paternity [53,54,73]. A more comprehensive discussion of important ethical challenges is outlined in the works of Professor Michael Parker [53].

8. Health economic perspective

The additional cost of prenatal exome sequencing, used alone, sequentially or in parallel with CMA, was evaluated following the PAGE study [74]. Performance of ES contingent on a negative CMA was the most cost-effective. At that time the cost of trio exome sequencing was quoted as £2100. The authors described an approach to ascertaining the wider costs associated with clinical care and ongoing management but could not examine the direct effect of a diagnosis on a pregnancy outcome and the ‘real life costs’ associated with termination of pregnancy or long term paediatric care [75]. Perception and calculation of ‘cost’, both financial and with respect to ‘harms’, varies between individuals and jurisdictions and there are currently no guidelines to assign a monetary value to a genetic diagnosis. Measures such as the quality-adjusted life-year (QALY) used to assess medical interventions in economic evaluations, are not suitable in these prenatal cases where termination of pregnancy or palliative care may be considered by parents as choices following a result.

Since the threshold at which the prenatal exome pathway can be deemed ‘cost effective’ is so challenging to determine, in the NHS the programme has started in a careful and limited manner. There is a higher diagnostic yield and therefore a lower cost per diagnosis in the subgroup of fetuses with multiple/multisystem anomalies, so the tests are applied to a subset of indications which will be progressively informed. This considered approach is believed to be the current best practice for resource allocation. A key workstream within the EXPRESS study will investigate the costs and cost-effectiveness of the rapid prenatal exome pathway, comparing to standard tests and to inform future design of an optimal testing pathway[41]. As more data become available, clinicians within healthcare systems will need to adjust the threshold at which the cost of solving a case (compared to restricting access to potential additional information until after birth) is deemed acceptable.

9. Perspective in the National Health Service England (UK)

Prenatal ES has been introduced into NHS-funded care in England since October 2020 [39]. The NHS-funded service has been nationally standardized and is delivered by a network of two central laboratories operating through specialist MDTs based in seven Genomic Laboratory Hubs (GLHs). As one of the testing laboratories and local MDTs we present our experience to date. Of 40 prenatal trio exomes performed both before and after national roll out, we achieved a diagnosis in 17/40 (42.5%) cases. These are cases selected according to specific criteria (Figure 1), selected by multidisciplinary review, where a monogenic malformation disorder is considered more likely. Since national roll out we have managed to report cases within an average of 12 days, and in many cases the result has influenced antenatal management and options open to couples in subsequent pregnancies.
Important learning from the initial phase includes the importance of the MDT for urgent communication around decision-making and the need for a robust protocol for referral, analysis and reporting. Detailed multi-source quality improvement will be provided by the EXPRESS study [41].

10. Conclusion

A prenatal exome can provide diagnostic information in previously unsolved cases of fetal structural anomalies, providing significant benefit over conventional karyotype and microarray analysis. There is a potential for discovery of incidental findings therefore robust protocols and counselling are important. A full appreciation of the prenatal exome test, and the availability of an expert Fetal Medicine / Genetics MDT to form the interface both with the expectant patient and the referring units is vital.

11. Expert opinion

Compared to the existing standard chromosomal/genetic testing, prenatal ES provides an additional diagnostic yield greater than 10% for unselected fetuses with structural differences. The yield increases to 20–30% or more after considered case selection of fetuses with multiple or multisystem anomalies. Prospective studies have already confirmed the clinical utility and acceptability of prenatal ES to clinicians and parents. Considerable laboratory and human resources are involved in the care pathway, which should be managed by an experienced multidisciplinary team comprising specialists in Fetal Medicine and Prenatal Genetics. The 'result' of the test is based on expert interpretation and moving forward all stakeholders can contribute to the future development of test utility by contributing to the scientific literature to enhance knowledge and share experience.

Future developments will depend on the pace of technological advances in the laboratory aspects of prenatal genetics. The use of CMA and ES is likely to be superseded by WGS in the future, as an 'all-in-one' test able to detect SNVs, CNVs and structural rearrangements. As cell-free fetal DNA-based testing develops further, ES for the fetus may be possible from a maternal blood sample. For some couples the risk of pregnancy loss associated with an invasive test is the only impediment in seeking genetic information, so the advent of a non-invasive test would likely improve the acceptability and uptake of prenatal ES.

Novel technology is being developed to avoid missing very subtle rearrangements currently not visible by karyotype. As the depth to which the fetal genome can be explored increases, the as yet uncharted territory generates much research interest. New discoveries relating to the non-coding region of the genome (for example enhancers, control regions, topological associated domains) will benefit patients but are as yet not fully understood. Whilst efforts to increase the diagnostic yield for monogenic disorders are important, it is also important to increase the evidence- and knowledge-base for the genetic diagnoses we already have.

The NHS rapid prenatal ES service is necessarily organised around centralised hubs with particular expertise and capability. It will be crucial that the referring units (the 'spokes' in the 'hub and spoke' model) receive ongoing education and feedback, to encourage collaboration and ensure equity of access throughout the healthcare system to this service which enhances prenatal care in pregnancies with complex fetal structural malformations on ultrasound.

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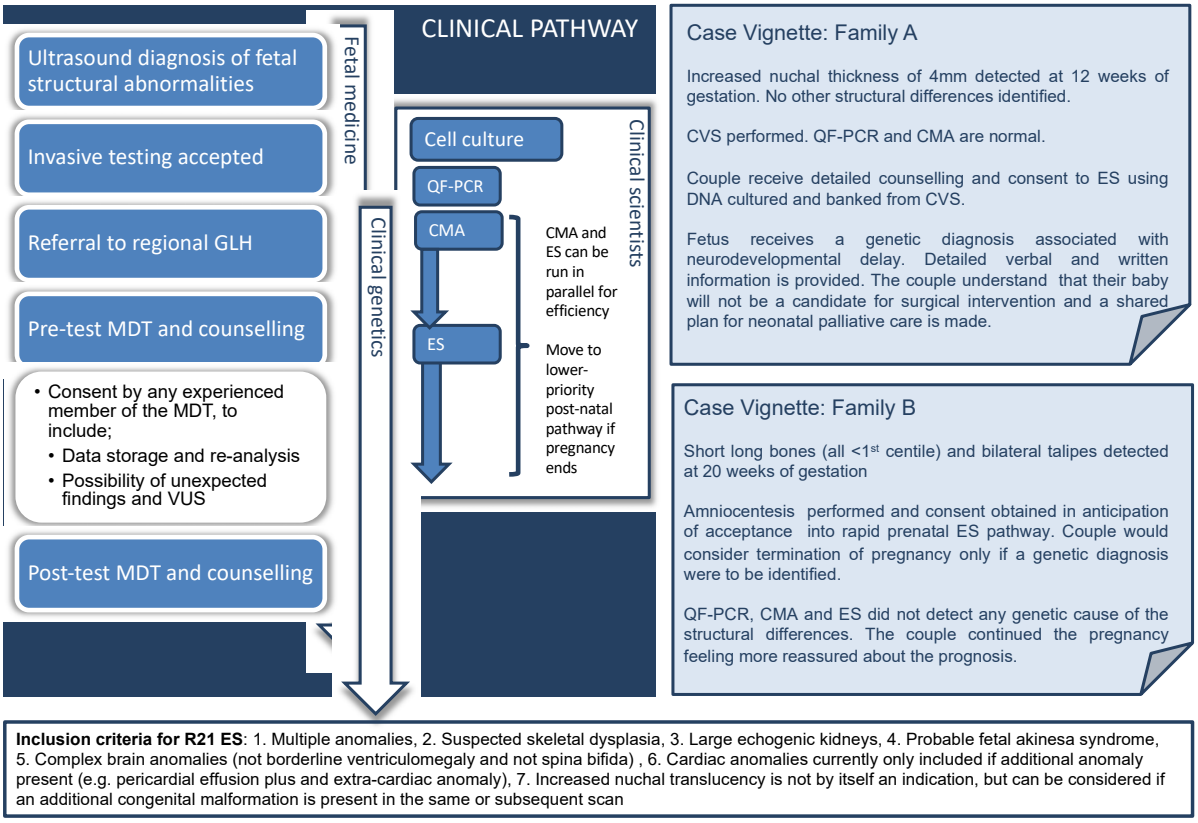
Table 1: Molecular diagnosis by phenotype

This table summarizes diagnostic yield data by ultrasound diagnosis. Highly selective studies selected by clinical geneticists or from postmortem reveal higher diagnostic rates compared to prospectively selected cases[5,18], although there is a paucity of data.

System	PAGE [18]	Petrovski et al[5]	Post Mortem cohorts	Clinical genetics cohorts	References
Skeletal	15.4% (10/65) Inc isolated talipes, transverse limb defects, etc	24% (8/34) single anomaly in 12/34	24% (n=20)	88% (2/26) 81% (13/16) 39% (28/72) (inc MSK)	[30] [64] [42] [
Cardiac	11.1% (9/81)	5% (4/77) single anomaly in 49/77	31% (n=26)	30% (11/37) (inc multisystem) 6% (n=50)	[29] [39] [30]
Craniofacial	3.1% (1/32)		21% (n=18)	46% (22/48)	[29] [30]
Hydrops	9.1% (3/33)	24% 5/21 single anomaly in 5/21		29% (37/127) Inc NT/cystic hygroma 60% (3/5)	[37] [65]
Brain	1.4% (1/69) Inc mild persistent and resolving VM	22% 11/49 single anomaly in 29/49		34% (22/65) (inc multisystem) 55% (5/9)	[29] [66]
Increased NT	3.2% (3/93)	12% (6/51)		7%	[37]
Renal	0% (0/14)	16% (4/25) (renal)	27% (n=23)	32% (12/38) 20% (4/20) bilateral echogenic kidneys 13% (4/30) All GU 9.1% (2/22) isolated CAKUT	[30] [29] [67]
Multisystem	15.4% (22/143) Multiple vs isolated anomaly *P*=0.019	19% (14/74) Multiple vs isolated anomaly *P*=0.005	38.5% (5/13)	31% 54%	[31] [29,34,35]

CAKUT (clinical abnormality of the kidney and urinary tract); GU, genitourinary; inc, including; MSK, musculoskeletal; NT, nuchal thickness; VM, ventriculomegaly

Figure 1: The NHS Rapid Prenatal Exome Pathway



Inclusion criteria for R21 ES: 1. Multiple anomalies, 2. Suspected skeletal dysplasia, 3. Large echogenic kidneys, 4. Probable fetal akinesa syndrome, 5. Complex brain anomalies (not borderline ventriculomegaly and not spina bifida) , 6. Cardiac anomalies currently only included if additional anomaly present (e.g. pericardial effusion plus and extra-cardiac anomaly), 7. Increased nuchal translucency is not by itself an indication, but can be considered if an additional congenital malformation is present in the same or subsequent scan