The role of genomics in investigating ultrasound identified fetal structural anomalies

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Abstract

Ultrasound-detected structural anomalies have an impact on fetal mortality and morbidity. Prenatal Exome Sequencing is incorporated into clinical care pathways for paediatric populations but maybe used to delineate the prognosis of fetal structural anomalies. This paper reviews the literature defining the clinical utility of prenatal ES and discusses the potential promise and challenges for implementation of this technology into clinical practice. Prospective case selection with accurate and informative pre-test counselling by multidisciplinary, clinical genetic-led teams is imperative. Robust, regulated laboratory sequencing, informative bioinformatic pathways with variant identification and conservative matching with the phenotype (within clinical review panels) is also important.

Existing prospective, prenatal exome sequencing studies.

The majority of studies describing the use of prenatal exome sequencing are small, retrospective 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 Chitty's group[9]. There were sixteen 'citations' which contained a range of inclusion criteria of babies with a structural anomaly were examined (including those associated with miscarriage, perinatal mortalityand termination of pregnancy). There were a range of isolated structural abnormalities , fetuses with multiple fetal abnormalities and those with an increased first trimester nuchal translucency (NT)(>3.5mm). Many reported fetus only)ES testing whilst others included 'trio testing' (fetus, mother and father), a method proven to increase diagnostic rate[10]. These papers reported a diagnostic rate that ranged from 6.2%[11] to 80%[12]. across sixteen studies[13-26]. However last year, two relatively large *prospective* -recruited cohort studies (from the United Kingdom and North America), targeting ES in fetuses with "unselected ultrasound" detected structural anomalies, were published in the Lancet, in early 2019[27,28]. A total 844 fetal probands (as eligible consented trios; when QF PCR and CMA was normal) were studied. In 76 probands (9%), a causative pathologic 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 identified a fetal anomaly and CMA was negative. Overall, ES provided an additional 8.5% diagnostic yield of pathogenic variants compared with conventional genetic testing. In addition to pathogenic variants considered to directly cause the relevant fetal structural anomaly, variants of uncertain significance (VUS) and of potential clinical relevance were diagnosed in a further 4% of cases[27]. The second prospective prenatal series conducted by Columbia University reported an additional diagnostic yield of 10.3% (in 234 trios)[28]. Both these studies gave diagnostic yields that were part lower than those reported from smaller retrospectively selected cohorts[9].

Fetal phenotype.

The two prospective studies recruited pregnancies in which the fetus had an ultrasound identified abnormality and the fetal medicine specialist believed was worthy of antenatal karyotyping (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[27,28]. The consequence of this was a heterogeneous mix of anomalies. Both studies recruited a high proportion of fetuses with multiple (>1) structural anomalies and the Petrovski study had a proportion of pregnancies included where there was a strong suspicion of 'genetic disease' (i.e. identification of skeletal dysplasia, cardiac rhabdomyoma; infantile polycystic kidneys)[28]. Both studies found a greater diagnostic rate of pathogenic variants in the presence of multiple congenital anomalies (between 15.4% - 18.9% respectively)[27,28]. This opens a debate as to whether to specifically select fetuses with specific single anomalies and/or greater than two major abnormalities for testing, and if testing should be broad (i.e. ES) 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[29], even with the use of additional modalities such as magnetic resonance imaging[30]. 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. Case selection of prenatally identified abnormal fetuses, after genetics review and additional phenotypic information from autopsy are two independent factors that increase diagnostic yield[31,32]. Published post-mortem series suggest a yield from ES of up to 30%, reflecting the importance of case selection, and detailed, accurate phenotyping (with the inclusion of subtle dysmorphic features)[10,23,33]. In addition, it is not just the identification of abnormalities that may be important. In childhood, the identification of neurodevelopmental and intellectual disability (not definable prenatally) may also indicate the potential underlying aetiology (Yang Y et al, 2013; DDD Study, 2015). The PAGE study identified specific phenotypes associated with the highest association of pathologic variants and 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 1). After correction for multiple testing, the detection of pathologic (or likely pathogenic) variants in a fetuses with multisystem anomalies was significantly more likely than in fetuses with any single abnormality (p=0.018)[27]. The Petrovski study demonstrated a similar association with structural phenotype; however renal anomalies (several fetuses with infantile polycystic disease) were associated with significant monogenic disease (16%)[28]. From the PAGE study, large nuchal translucencies (>4mm) had a relatively low association with identifying a pathologic variant (unless on subsequent scanning at 20 weeks additional anomalies were identified)[34].

It should also be recognised that prenatal and postnatal phenotypes may differ with the same underlying genetic pathologic variant. A good example of this is in the presence of mutations in the histone methyltransferase KMT2D and the demethylase KDM6A (of which multiple variants are known)[35] being associated with Kabuki syndrome. In childhood, this is characterised by classic facial gestalt, multiple organ malformations, abnormal postnatal growth and intellectual morbidity[36]. In prenatal life, it appears associated with nuchal oedema, hydrops fetalis, cardiac malformations, intrauterine growth restriction and stillbirth[27,28].

Bioinformatics and interpretation of variants identified using ES.

In the PAGE study, sequenced data were assessed for candidate pathogenic variants from a modified gene list of genes (DDG2P list (1628 genes)[27]) that were likely to be associated with developmental disorders[37]. 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 bioinformative filtering). These 'candidate pathologic variants' were then fed onto and reviewed by the clinical review panel (CRP). The CRP (a multidisplinary group of clinical geneticists, fetal medicine subspecialists, two clinical scientists and a genetic bioinformaticians) 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. pathologic, likely-pathogenic, variant of unknown significance), likely benign and benign) and the likelihood that it was causative of the fetal phenotype. Using this methodology, 0.4 variants were reviewed per proband (fetus with structural anomaly)[27].

The Petrovski paper used similar but not identical methodology for identification of pathologic variants[28]. Again they used trio analysis and a previously published framework[to] allow rapid and efficient identification of de-novo and inherited variants[38]. This focused upon two 'tiers' of qualifying genotypes. Tier 1 were 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 amongst controls (internal and external) and had to have been previously classified a pathogenic on *Clinvar* or Human Gene Mutation databases[28,39]. Again potential causative variants were classified by a multidisplinary 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 to PAGE (4.8 variants per case versus 0.42 per case requiring manual interpretation by PAGE), with a limited difference in the overall final pathogenic variant yield[28]. This demonstrates the need to balance a higher diagnostic yield versus 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 may be updated with time. This means that if the fetal trio ES were to be periodically reanalysed (during childhood) every 1-2 years additional pathologic variants may 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 (VUS) 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 were termination of pregnancy is an option). Parents report that such information is 'toxic' and emotional effects may last for a considerable period[41,42,43].Therefore, as prenatal ES is used to evaluate congenital malformations in clinical practice there is a need to register 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"[9]. In a paediatric setting, the American College of Medical Genetics and Genomic (ACMG) has set out guidelines that state when offering ES, secondary findings should be reported in 59 genes (in which it is believed that there is clinical evidence that pathologic variants may result in disease that may be prevented or treated)[44]. However, in 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[45]. An example of this would be the potential ES testing of a fetus with a phenotype suggestive of Fanconi anaemia[46]. Somatic inactivation of the Fanconi anaemia /BRCA pathway accounts for the chromosomal instability the predisposition of some cancers (breast, bowel, and ovary) in the general population[47]. However, it is recommended that if instituted it should be presently on a case by case basis and pre-and post-test counselling of parents is careful and detailed before this is instituted. In addition, trio ES could also reveal unforeseen issues such as non-paternity or parental consanguinity again leading to difficult counselling scenarios[48,49].

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 and when prenatal invasive tests are contemplated it is the obstetrician who usually discusses the procedure and laboratory test to the parents. In North America, this role is often taken up by the genetics team, commonly the specialist genetics nurse. 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, sensitivity and specificity of diagnosis (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[9]. 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* [50]. If the multidisciplinary team (comprising the same group of specialists as used in the PAGE study)[27] decide that the pregnant women 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[9,50].

Once the laboratory based ES and potential variants have undergone bio-informative filtering, then a multi-disciplinary 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(ies). Once a causative genetic variant has been identified, this again, needs to be discussed with the parents within a multidisciplinary clinic with the clinical genetic team explaining clinical relevance, potential inheritance and any further testing (especially of family members). Such complex clinical pathways and infrastructure are starting to be developed in the UK and multidisciplinary research (through the Optimising **EX** ome**PRE** natal **S** equencing **S** ervices [EXPRESS study]) is underway to aid the development of such pathways and to ensure equity of access and high clinical standards across England[51].

Clinical pathway 'turnaround time',

In the PAGE study, an *à priori* protocol decision was made only to feedback information on pathologic variants after the end of the pregnancy[27], whereas the Petroski study (indicated 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[28]. 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 provide clinical benefit, improved decision making, aiding developmental and family emotional outcomes[8,52,53,54]. In prenatal diagnosis there is also a requirement for a relatively rapid TAT to aid informed parental decision making. Such 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 panels assessment). Normand and colleagues indicated that prenatal TAT could be achieved on average within 14 days (range 7-38 days)[32]. 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 were within 21 days[55]. Certainly, parents (pre-testing) must be aware that test TAT can be of variable duration but that on average a result should be obtained within 28 days (Dr Dom McMullan personal communication, March 2020).

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[9]. 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[56]. Our own, rather conservative cost modelling used 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. 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 the stepwise 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 the stepwise would cost a further additional GBP 11,639 (EUR 13,340/USD 14,929) per additional genetic diagnosis. The sub-group analysis suggests that performing stepwise on cases indicative of multiple

anomalies at ultrasound scan compared to cases indicative of a single anomaly, is more cost-effective compared to using ES alone[57]. It is likely that these are conservative costs and different health care economies will need to evaluate their costs and make decisions as to whether it is possible to implement prenatal exome sequencing within their health care systems.

The potential implementation into the prenatal diagnostic pathway in England.

A national NHS Genomic Medical Service infrastructure based around seven laboratory hubs across England is in place. It is envisaged that prenatal case selection will be by a multidisciplinary (tertiary) team, led by a clinical geneticist. Phenotype selection is paramount and is likely to focus upon fetuses with: i) multiple anomalies, ii) severe skeletal dysplasia, iii) non-immune hydrops or iv) structural anomalies where there is a strong suspicion of genetic aetiology (i.e. infantile polycystic renal disease; complex CNS anomalies and fetal arthrogryposis/fetal akinesia syndrome). The laboratories charged with providing prenatal ES would use a modified panel of pathologic variants identified in the PAGE study (DD-G2P/PAGE), using a traffic light rating and comprising 90-100 variants that have been chosen after expert review and agreed by NHS Genomic Medical Service sign off (https://panelapp.genomicsengland.co.uk/panels/478/)[58]. As indicated in early discussion within this review, the clinical infrastructure for delivery of this prenatal service needs to be conservative but robust. The desirable clinical and laboratory infrastructure required will be informed by prospective, NIHR-funded research through the EXPRESS study[51]. However, it is probable that both phenotypic inclusion, the pathologic variant list and the clinical infrastructure for delivery will remain under continuous review and there is a recognised need for the formal curation of pathologic, probable pathologic and VUS variants matched to detailed phenotyping within a database such as *ClinVar* [39]. It is also likely that a strategy for re-testing will be requested as noted to be important in paediatric testing strategies[40].

Conclusion.

The implementation of prenatal ES into clinical practice to evaluate the fetus with structural anomalies provides an 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 pathologic 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[59]. 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 whilst attempting to limit an unnecessary emotional burden to parents and the criticism of societal eugenics[48,49,60].

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