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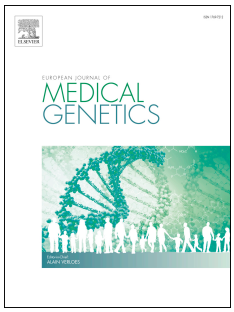

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PII: S1769-7212(19)30110-7

DOI: https://doi.org/10.1016/j.ejmg.2019.05.002

Reference: EJMG 3663

To appear in: *European Journal of Medical Genetics*

Received Date: 8 February 2019

Revised Date: 26 April 2019

Accepted Date: 4 May 2019

Please cite this article as: L. Ferretti, R. Mellis, L.S. Chitty, Update on the use of exome sequencing in the diagnosis of fetal abnormalities, *European Journal of Medical Genetics* (2019), doi: https://doi.org/10.1016/j.ejmg.2019.05.002.

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Update on the use of exome sequencing in the diagnosis of fetal abnormalities

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Abstract

Unexpected fetal abnormalities detected through ultrasound scanning in pregnancy may have a monogenic aetiology but are difficult to diagnose. Next generation sequencing now enables us to sequence fetal exomes, providing increased resolution and broader diagnostic capability compared to traditional cytogenetic prenatal tests, improving the yield and accuracy of diagnoses and allowing better counselling for expectant parents.

Here we review published studies of exome sequencing (ES) for prenatal diagnosis over the last 5 years and address important questions for its clinical implementation, including clinical utility, which groups benefit most, and practical and ethical challenges for interpreting and reporting results.

We observe that fetal ES substantially improves diagnostic yield relative to cytogenetic techniques. However, diagnostic rates vary widely between studies, largely attributable to differences in case selection. Recently several large studies report variations in diagnostic yield between phenotypic groups, with fetuses with multisystem abnormalities most likely to receive a diagnosis from fetal ES. Challenges for prenatal ES include the limitations of ultrasound-based fetal phenotyping, the need for rapid return of results in pregnancy, and technical limitations compared to whole genome sequencing. We also consider ethical issues around potential secondary findings and variants of uncertain significance and the complex counselling needs these present.

Prenatal ES is a valuable tool to diagnose fetal abnormalities and, as it is implemented in the clinic, more large-scale research will serve to further delineate its clinical utility, as well as

generating new knowledge about fetal phenotypes and informing guidelines for case selection, reporting results and genetic counselling.

Key words: Prenatal, Exome sequencing, Fetal structural abnormalities, Monogenic disorders

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Introduction

The armory of technologies available to us for making prenatal genetic diagnoses in fetuses has expanded rapidly over the past few years. In the UK, pregnant women routinely receive a dating scan between 10 and 14 weeks gestation and a fetal anomaly scan between 18 and 21 weeks to detect any fetal abnormalities. During the dating scan, nuchal translucency (NT) is measured which informs risk calculations for Trisomy 21 and other aneuploidies. Unexpected fetal anomalies occur in up to 2-3% of pregnancies and many are caused by an underlying genetic disorder. A definitive genetic diagnosis in pregnancy has many potential benefits, including better-informed counselling of parents regarding prognosis, thereby empowering parental choice. Where prognosis is known to be poor, this might involve offering the option of termination of the pregnancy; where prognosis is good, this provides reassurance and enables continuation of the pregnancy. Increasingly, accurate molecular diagnosis will allow for targeted *in utero* or early postnatal treatment including delivery plans and preemptive involvement of specialist paediatric services. A genetic diagnosis also informs reproductive decision-making and the management of future pregnancies and finally, may have implications for other family members.

Families who choose to further investigate abnormal sonographic findings in pregnancy have traditionally been offered one of two invasive fetal sampling methods: amniocentesis and chorionic villus sampling (CVS). Amniocentesis is ideally performed after 15 weeks gestation and is quoted to carry up to a 1% risk of miscarriage, although recent evidence suggests that this risk is in fact closer to 0.1% (Akolekar et al., 2015). CVS can be performed earlier, from 10

weeks, and is traditionally said to carry a slightly higher risk of miscarriage than amniocentesis; however, recent studies suggest this risk is not significantly different to controls (Akolekar et al., 2015; Odibo et al., 2008; Tabor et al., 1986). Analysis of the fetal genetic material by rapid quantitative fluorescence polymerase chain reaction (QF-PCR), karyotyping and chromosomal microarray (CMA) will detect aneuploidies, large chromosomal rearrangements and pathogenic micro-deletions and duplications, identifying in between 27.4% of underlying aetiologies where abnormal ultrasound findings is the indication, and 40.7% when including other clinical indications for testing (Fiorentino et al., 2013). However, the remaining majority of fetuses with sonographically detected abnormalities are left without a diagnosis. Some of these fetuses will have an underlying monogenic disorder, but prenatal diagnosis in the absence of a family history has traditionally been challenging, costly, time-consuming and thus not practical in the timeframe of a pregnancy. The evolution of next generation sequencing (NGS) has allowed for broader testing in this setting by enabling screening of multiple genes in a single analysis, where previously to diagnose a genetic problem arising *de novo* would potentially have required screening many genes sequentially.

Sequencing approaches range from targeted sequencing using phenotype-specific gene panels which can be confined to tight criteria, for example a 240 skeletal gene panel, or a much broader 'clinical' panel which can include up to 6000 known disease-causing genes, through to whole exome sequencing (WES) and whole genome sequencing (WGS). The latter can then be analysed using selected panels for a more targeted approach. The exome represents 1-2% of the genetic code but contains approximately 85% of known disease-related variants. Exome

sequencing (ES) improves upon the current standard techniques used prenatally (CMA and karyotyping) with its increased resolution, ability to detect changes down to the single base pair and thus identify pathogenic variants in disease-causing genes. While ES will not detect intronic variants which may affect splicing for example, the use of ES both postnatally in dysmorphic children and prenatally in anomalous fetuses with a normal karyotype or microarray significantly increases diagnostic yield (Best et al., 2018; Wright et al., 2015). From a practical standpoint, using a targeted approach either for sequencing or analysis will limit diagnosis to known genes. Whilst this approach is therefore not useful from a research perspective in terms of new gene discovery, it does avoid many of the issues around reporting of uncertain or secondary findings, discussed in more detail below. Furthermore, where ultrasound findings do not correlate with a specific phenotypic group, a broader approach such as WES or a large clinical panel covering genes likely to present prenatally is likely to be a more efficient approach to prenatal diagnosis.

Over recent years there have been many publications showing the diagnostic power of ES for the diagnosis of fetal structural abnormalities (Best et al., 2018). Many of these reports are of single cases or small, highly selected series of cases, with results largely reported back to parents after pregnancy ends. When considering series of more than 10 cases (**Table 1**), diagnostic rates of up to 80% have been reported. Very recently, at the end of 2018 and early in 2019, three larger series have been reported (Lord et al., 2019; Normand et al., 2018; Petrovski et al., 2019). These confirm the increased diagnostic power of ES, but in unselected cohorts the overall diagnostic yield was 8.5% and 10.3% (Lord et al., 2019; Petrovski et al., 2019). Thus,

there remain many questions to be answered including determination of clinical utility and which groups of fetuses would benefit most. This is particularly pertinent as the cost remains high and trio sequencing is required to maximise the diagnostic yield thus resources must be allocated judiciously. Furthermore, we must address the suitability of ES given it does not cover the whole genome; the efficacy of prenatal phenotyping based on ultrasound scanning alone; whether fetal phenotypes are the same as those we see postnatally and how to account for prenatally lethal conditions where the phenotype may be less well recognised. There are also unique challenges around reporting sequencing results in the prenatal setting, particularly as we are likely to be sequencing parents as well as fetuses. This makes fetal ES a complex test to convey to parents and necessitates careful consideration: what levels of counselling will be required, who should do it and is it needed both before and after testing?

In this review we describe the current state of the art for prenatal ES and discuss the potential benefits, challenges and ethical issues that might arise when implemented clinically.

Reference	**No. of probands**	**Inclusion criteria**	**Method**	**Pathogenic variants**	**Likely pathogenic variants**
Petrovski et al., 2019	234	Fetuses with structural abnormalities on USS and normal karyotype/CMA	WES in trios	24 (10.3%)	46 (19.7%)
Lord et al., 2019	610	Fetuses with structural abnormalities/increased NT and normal karyotype/CMA	WES 596 (97.7%) in trios 14 (2.3%) in dyads	52 (8.5%)	24 (3.9%)
Normand et al., 2018	146	Live and terminated/miscarried fetuses with abnormalities on USS	WES 95 (65%) in trios	46 (32%): 29 (20%) in trios 17 (12%) in	Not specified

				singletons	
Fu et al., 2018	196	Stored samples from live fetuses with structural abnormalities on USS/MRI and normal karyotype/CMA	WES 49 (25%) in trios	47 (24%): 13 (26.5%) in trios 24 (23.1%) in singletons	Not specified
Chandler et al., 2018	16	Fetuses with USS abnormalities where expert MDT review considered skeletal dysplasia likely	WES in trios	13 (81%)	1 (6.25%)
Leung et al., 2018	33	Lives fetuses with abnormalities on USS and normal CMA	WES 31 (94%) in trios/quadruplets	3 (33%)	Not specified
Zhou et al., 2018	12	Fetuses with sonographic features suggestive of a skeletal dysplasia	Proband only targeted skeletal panel ES	8 (67%)	2 (17%)
Ryan et al., 2017	129	Fetuses with USS abnormalities	WES in trios (78) and singletons/other family combinations	32 (25%) 21 (26.9%) in trios only	69 (53.5%)
Yates et al., 2017	84	Terminated or miscarried fetuses with USS abnormalities	WES 51 (61%) in trios/quads	17 (20%): 11 (24%) in trios 14 (4%) in singletons	38 (45%)
Joset et al., 2017	60	Live and terminated fetuses with USS abnormalities	'Mendeliome' (extensive gene panel) and WES (unspecified)	18 (30%)	Not specified
Lei et al., 2017	30	Live fetuses with CAKUT and normal karyotype/CMA	WES 7 in trios	4 (13%): 1 (14%) in trios 3 (13%) in singletons	Not specified
Vora et al., 2017	15	Fetuses with abnormal ultrasound findings but normal routine genetic investigations.	WES in trios	7 (47%)	1 (6.7%)
Sa et al., 2017	15	Terminated or miscarried fetuses with USS abnormalities or multiple	Clinical exome in 13 (86.7%) WES in 2 trios	8 (53.3%)	1 (6.7%)

		abnormalities on autopsy	(13.3%)		
Walkiewicz et al., 2016	61	Carefully selected cases of fetuses with ultrasound abnormalities and normal karyotype/microarray.	WES 21 (34.4%) in trios 40 (65.6%) in singletons	20 (32.8%) 8 (38.1%) in trios and 12 (30.8%) in singletons	Not specified
Pangalos et al., 2016	14	Live and terminated fetuses with abnormalities on USS and normal CMA	Targeted exome sequencing in singletons	5 (36%)	2 (14%)
Drury et al., 2015	24	Live fetuses with increased NT measurement/abnormalities on USS and normal karyotype/CMA	WES 10 (42%) in trios	5 (21%): 3 (30%) in trios 2 (14%) in singletons	1 (4%)
Carss et al., 2014	30	Live fetuses and newborns with abnormalities on USS and normal karyotype/CMA	WES in trios	3 (10%)	5 (16.7%)
Yang et al., 2014	11	Terminated fetuses with USS abnormalities	WES in trios	6 (54.5%)	Not specified

Table 1. Case series of WES used to diagnose fetuses with abnormalities detected on prenatal USS since 2014 to the present day. Series where N</=10 have been excluded. Adapted from Best et al. 2018 with permission.

WES – whole exome sequencing; ES – exome sequencing; USS – ultrasound scanning; CMA – chromosomal microarray; NT – nuchal translucency; MRI – magnetic resonance imaging; CAKUT – congenital anomaly of the kidney/urinary tract

Clinical utility

Multiple examples from recent studies highlight the clinical utility of fetal ES where routine prenatal testing with karyotype and CMA did not yield a diagnosis. For instance, we have encountered a case of a pregnancy presenting at 16 weeks gestation with fetal ultrasound findings of slightly shortened long bones, bowed femurs and humeri and mild bone hypomineralisation, suggestive of a skeletal dysplasia. The suspected clinical diagnosis based on ultrasound scan (USS) findings alone was osteogenesis imperfecta, a heterogeneous condition

with variable inheritance, although most are *de novo* autosomal dominant conditions. Trio ES revealed fetal homozygosity for a pathogenic variant in *LEPRE1* (c.1080+1G>T), giving a genetic diagnosis of osteogenesis imperfecta type VIII, an autosomal recessive condition with bi-parental inheritance of the variant. This not only facilitated accurate prenatal counselling regarding prognosis, but also informed future reproductive decisions as the parents could be counselled regarding 25% recurrence risk and offered molecular prenatal diagnosis in future pregnancies. A further example reported by Drury *et al*. (2015) used ES in a biological trio where the fetus had increased NT and pedal oedema, to identify a maternally inherited variant in *FLT4* (c.3075G>A p.Met1025Ile) causing Milroy syndrome. Further careful interrogation of the family history revealed that the affected mother had had self-resolving pedal oedema at birth but had not previously been aware of this. Thus, the molecular diagnosis permitted reassurance regarding prognosis for the affected baby, as well as accurate counselling regarding future pregnancies. The same group reported a paternally inherited pathogenic variant in *MYH3* (c.2014C>T p.Arg672Cys) in a 25 week gestation fetus with clenched hands, neck flexion and micrognathia on ultrasound. This is known to cause Freeman-Sheldon syndrome which demonstrates autosomal dominant inheritance. The proband's father also had a small jaw but no evidence of or history of contractures or 'athrogryposis', the genetic causes of which are varied and can present a 'diagnostic odyssey' when discovered at birth. Chandler *et al*. (2018) reported a case of short, angulated bones in a fetus suggestive of a skeletal dysplasia. Rapid trio ES revealed a maternally-inherited variant *ALPL* (c.331G>A p.Ala111Thr) giving a genetic diagnosis of autosomal dominant hypophosphatasia in both the fetus and mother, who had

short stature only. This too provided a reassuring diagnosis for the family who decided to continue the pregnancy.

Which groups may benefit most?

Of the studies described in **Table 1**, some took a very inclusive approach where WES was performed on any fetus with anomalous ultrasound scan findings, whereas others took a curated cohort in whom a genetic diagnosis was considered likely following expert review. It is notable that dramatically higher diagnostic yields were achieved in those highly selected cohorts. For example, diagnostic rates of >80% have been achieved by selecting fetuses in which there was a likely skeletal dysplasia, as judged by experts in fetal medicine and genetics (Chandler et al., 2018; Zhou et al., 2018). Similarly, Walkiewicz *et al*. (2016) and Byrne *et al*. (2017) achieved diagnostic rates of 32.8% and 50% respectively by selecting families in which there was a history of parental consanguinity, large regions of homozygosity on SNP array, multiple malformations or unexplained miscarriage or neonatal death.

In contrast, the Prenatal Assessment of Genomes and Exomes (PAGE) study recently ended in the UK having used prenatal WES in a large cohort of unselected fetuses with structural anomalies detected on ultrasound scanning and a normal karyotype and CMA (Lord et al., 2019). Here, any fetal anomaly was eligible for inclusion; including isolated raised NT, isolated mild ventriculomegaly, isolated talipes etc., and cases were not subject to expert genetic review prior to recruitment. Interpretation of WES data was targeted to a virtual panel of genes deemed relevant to conditions which can present prenatally. Trio sequencing and analysis was

undertaken in the majority of cases and only pathogenic variants relevant to the observed fetal phenotype based on ultrasound findings were reported to parents, after the pregnancy ended. The overall diagnostic rate of 8.5% reported in this study of 610 fetuses is lower than previous smaller studies of fetal WES, despite using trio analysis. This is likely to be a result of the unselected nature of the cohort studied and thus may inform guidance on which groups of fetal abnormalities to target in clinical practice. Of note, where the only abnormality detected is increased NT, diagnostic rates appear to be much lower; at 3.2% in the PAGE study and with other studies reporting no pathogenic findings in this group (Daum et al., 2019).

When subgroups in the PAGE study were analysed it was found that diagnostic rates were highest in fetuses with multisystem abnormalities (15%), skeletal anomalies (15%), or cardiac anomalies (11%) (Lord et al., 2019). These findings are in agreement with those of Petrovski *et al.* (2019) who performed a similar study of trio WES in 234 unselected fetuses. They reported an overall diagnostic rate of 10.3% with a higher rate in fetuses with anomalies affecting multiple systems (18.9%) and those with lymphatic (24%), skeletal (24%), central nervous system (22%) or renal (16%) anomalies. They also found that the rate of monogenic diagnoses increased with the number of anomalies present. Other fetal ES studies also report their highest diagnostic rates in fetuses with multisystem abnormalities or in specific phenotypic groups such as craniofacial anomalies or dysmorphic facial features (Fu et al., 2018; Normand et al., 2018). However, notably even in the larger series reported, the numbers of fetuses in any particular system group are small and further analysis of larger cohorts will be required before

drawing any definitive conclusions as to which inclusion criteria yield the highest diagnostic rates (Lord et al., 2019; Petrovski et al., 2019).

Nonetheless, it appears broadly that diagnostic yield is increased by the careful selection of cases where multiple fetal abnormalities are present or where clinical genetic review suggests a higher likelihood of an underlying genetic disorder. Better diagnostic yields are also achieved through sequencing trios of fetus, mother and father together compared to sequencing singleton fetuses. Trio analysis is more efficient for making a diagnosis because it allows for the rapid identification of *de novo* variants and informs whether variants within the same gene are present in *cis* or *trans*. Therefore, selecting cases where trio analysis is possible will also help to maximise diagnostic rates, as well as to expedite analysis and reporting of results. This does, however, contribute to the relatively high cost of testing.

One other group of patients that may benefit from WES for prenatal diagnosis are parents who have had a previous affected pregnancy where an autosomal recessive condition is likely, but no definitive diagnosis was made, and no stored DNA or tissue is available for testing. Under these circumstances, parental sequencing has been shown to identify heterozygous pathogenic (or likely pathogenic) variants in more than 50% of couples to give a genetic diagnosis in the deceased proband, with the diagnostic yield increasing when two or more fetuses were affected. The authors concluded that parental ES is a powerful strategy for the genetic diagnosis of lethal or prenatal-onset recessive disorders (Stals et al., 2018).

Developing a rapid pipeline

Many of the studies described in **Table 1** used DNA from terminated or miscarried fetuses as well as ongoing pregnancies. The use of WES in terminated fetuses has less relevance in terms of obtaining a clinically timely prenatal diagnosis but is useful in the research setting and for counselling parents about risk of recurrence in future pregnancies. Most studies to date, including the PAGE study, did not return diagnostic results in time to be actioned during pregnancy (Lord et al., 2019). Nonetheless, PAGE did provide an early diagnosis in conditions which can take many years to diagnose postnatally due to the delayed manifestation of distinctive features; of note, pathogenic variants in *KMT2D* provided a timely diagnosis of Kabuki syndrome in fetuses with variable, non-specific ultrasound anomalies. Antenatal diagnosis is time-limited and the earlier a result is obtained, the more useful it is for ongoing management of the pregnancy. For this reason, a rapid pathway and pipeline is essential to optimise the utility of such a service in clinical practice. **Figure 1** demonstrates the workflow developed by Chandler *et al*. to facilitate rapid return of results to patients. This involved simultaneous sequencing of trio samples restricted to a 'clinical exome' of 20Mb, the use of a rapid bioinformatic pipeline for sequence alignment, variant calling and annotation, and targeting of the analysis to a phenotype-specific gene panel (Chandler et al., 2018). This pathway is currently being used in our laboratory to diagnose skeletal dysplasias in fetuses with suggestive ultrasound findings with an overall diagnostic rate of 86% and average turnaround time of 10-12 days from the samples being received in the laboratory to issuance of a report (unpublished data). Of note, multidisciplinary review was employed for both case selection and for variant interpretation.

In the report from Normand *et al*., retrospective review of ES results derived from one of three different protocols undertaken by Baylor Genetics clinical diagnostic laboratory was reported (Normand et al., 2018). One of these protocols has a rapid 2-3 week turnaround time for prenatal trios, where urgent establishment of a diagnosis *during* pregnancy is desirable. Parental and fetal samples were sequenced simultaneously and analysed as one dataset. The diagnostic rate achieved with the rapid protocol (35%) was not significantly different to that achieved with the proband-only ES (33%) (12.6 week turnaround time) and the standard trio ES (21%) (6.2 week turnaround time) protocols, demonstrating that rapid return of results can be achieved without detriment to diagnostic yields. These turnaround times did not include time required for tissue culture when it was necessary before DNA extraction but did include confirmation of the likely causative variant using Sanger sequencing in an accredited diagnostic laboratory.

FIGURE 1 HERE

Incomplete coverage and other limitations

Whilst it has been demonstrated that WES clearly has value in the prenatal setting, it is not without its drawbacks. These include the limitations of WES itself, which targets only the coding region of the genome. Some regions, particularly CpG islands, will be poorly covered due to techniques used to capture the exome (Belkadi et al., 2015; Meienberg et al., 2016). This can have a detrimental effect on obtaining a definitive genetic diagnosis. There are several

examples in the literature of one mutation being identified in a recessive gene compatible with the fetal phenotype, but no second mutation being identified. For example, in a 20 week fetus with severe ventriculomegaly, atrioventricular septal defect (AVSD), polydactyly and echogenic kidneys, a single heterozygous paternally-inherited variant in *DYNC2H1* (c.4261-2A>G) was identified (Drury et al., 2015). Despite high depth panel sequencing of the gene in question (variants in which are known to be associated with autosomal recessive ciliopathies), only 87% coverage of the gene was achieved on both ES and high depth sequencing. A second variant in the gene has therefore not been found and the family remain without a definitive molecular diagnosis. Post-mortem examination confirmed the sonographic findings and was suggestive of a ciliopathy, but the family could only be offered an empirical recurrence risk of 25% based on the assumption that an undetected second maternally-inherited variant was present.

The coverage of these areas is improved with WGS, as is the ability to detect intronic variants, structural variants, trinucleotide repeats and large deletions/CNVs (Belkadi et al., 2015). However, for the current greater cost of WGS over WES, the benefit in terms of diagnostic yield is not yet well-evidenced. It has been demonstrated that by simply re-analysing data in 38 WES-negative cases, a further 23 diagnoses were made either through use of more refined bioinformatics pipelines, utilising more accurate phenotyping data or because new phenotype:genotype associations had been reported since the original analysis (Shashi et al., 2018). In this same study, WGS only revealed an additional three diagnoses (Shashi et al., 2018). In another study, re-analysis of WES results using a combination of prenatal and postnatal phenotyping yielded pathogenic variants in around 20% of cases previously undiagnosed

(Filges and Friedman, 2015). These findings highlight how a lack of accurate prenatal phenotyping, based solely on ultrasound findings, presents a challenge and may also contribute to lower diagnostic rates.

New and evolving phenotypes

As discussed above, prenatal phenotypes are limited by the constraints of imaging and lack of developmental assessment/post-natal investigations, which can be problematic when assigning pathogenicity to variants. Fetal phenotyping can be enhanced to some extent by using more sophisticated imaging techniques such as fetal MRI and 3D/4D ultrasound scanning, but some phenotypes such as intellectual disability cannot be assessed prenatally. In addition, we may see new phenotypes associated with genes not previously reported in the prenatal period, or identify phenotypes we would not necessarily associate with the condition we recognise postnatally. For example, Sotos syndrome is associated with deletions in *NSD1* and in the postnatal period is recognised as an overgrowth syndrome.

However, Zhang and colleagues reported a fetus with poor intrauterine growth only and an *NSD1* deletion, indicating that phenotypes may evolve over time (Zhang et al., 2017). In this case the pregnancy was terminated so it was not possible to determine whether or not the phenotype might evolve. We have subsequently encountered a similar case locally of a fetus presenting with poor intrauterine growth where ES detected a pathogenic missense variant in *NSD1* and at one year of age the infant had developed macrocephaly and clinical features of Sotos syndrome (unpublished data). Petrovski and colleagues encountered a similar diagnostic challenge in their study, where a fetus with isolated increased nuchal translucency was found to

have a de novo frameshift variant in the *RERE* gene (Petrovski et al., 2019). Pathogenic variants in this gene are associated with a neurodevelopmental disorder with variable structural abnormalities of the brain, eye and heart, so based on the information available during pregnancy the team could not definitively associate this variant with the fetal phenotype. It was only after 6 months postnatally that the infant was found to have other features consistent with a *RERE*-related disorder and the variant could be re-classified as pathogenic, signifying a novel presentation of the disease prenatally with isolated increased nuchal translucency. These examples add weight to the concept of previously unrecognized evolving prenatal phenotypes in postnatally recognized disorders.

In addition to identifying new fetal phenotypes for known genetic conditions, the phenotypes of conditions not previously reported are being identified. For example, in the PAGE study a number of different cases were reported which were the first instances of prenatal identification of mutations in their respective genes (Lord et al., 2019). The increasing identification of these new and evolving fetal phenotype:genotype associations as prenatal ES becomes more widely used means data-sharing is key to our understanding and will ultimately improve our ability to interpret results. Understanding of genes involved in prenatal development remains poor and further research is also required to elucidate the function of these genes in the setting of lethal fetal phenotypes (Filges and Friedman, 2015).

These studies suggest that incomplete prenatal phenotyping, recognition of new prenatal phenotype:genotype associations and lack of prenatal ultrasound:genotype databases may be

limiting factors for current interpretation of WES data in prenatal diagnosis. Development of internationally shared prenatal phenotype:genotype databases would significantly improve WES interpretation in this setting. Further, these difficulties with interpretation raise the issue of whether and when to re-analyse or re-sequence when negative results are obtained, since these could change in light of new information.

Reporting challenges and ethical issues

The ethical debate regarding reporting of WES in the prenatal setting does not differ greatly from that which already exists for genetic testing using NGS for diagnosis in children (Horn and Parker, 2018). Issues of autonomy for the unborn child and reporting of secondary or incidental findings remain, yet ACMG guidance on reporting clinically significant secondary findings specifically excludes prenatal samples (Green et al., 2013). The likelihood of a clinically significant secondary finding with WES is higher than with karyotyping or microarray due the greater depth of analysis and the scale of data obtained, and will be present in at least 1 in 200 individuals (Amendola et al., 2015). In the setting of trio analysis, secondary findings may be detected in the parental as well as the fetal genotypes. Whilst the ACMG guidance may be applied to the parents when consenting and returning results of secondary findings such as cancer predisposition syndromes and familial hyperlipidaemia, if the parents are permitted to consent for return of secondary findings in the fetus, this then compromises the child's autonomy and future right to decline to receive such results. However, it is evident from studies in the USA and the UK that parents are keen to have all possible information regarding their child's health, whether it is related to the specific problem detected in pregnancy or not,

with the significant majority in the USA wanting to know about treatable and non-treatable childhood (>85%) and adult onset (>74%) conditions (Kalynchuk et al., 2015; Quinlan-Jones et al., 2017). Incidental findings such as non-paternity and consanguinity are not usually reported but may be important in the context of trio analysis and maximizing the chance of obtaining a diagnosis. Parents need to be aware that such findings may be identified before testing is undertaken.

The other group of findings which are a matter for discussion are variants of unknown significance (VUS) - those variants for which there is insufficient evidence to classify them as either pathogenic or benign. Uncertainty surrounding these findings is considerable and may cause parental anxiety without a clinical benefit. However, the classification of these variants may evolve as new evidence emerges and so it is important to establish what, if any, system will be in place for re-classifying variants over time or whether it would be more cost-efficient to re-sequence at a later date in such cases (Abou Tayoun et al., 2018). There is disparity between the views of clinicians and patients with regard to this issue, as reported by Quinlan-Jones *et al*. who observed that patients feel strongly that results should be reviewed over time, whilst clinicians feel that this is practically not feasible with current resources and availability of adequately educated genetic counsellors (Quinlan-Jones et al., 2016). The International Society of Prenatal Diagnosis (ISPD) recommends that re-analysis or re-sequencing is best undertaken if a specific need arises, for example a new pregnancy is planned or the phenotype evolves significantly, rather than undertaking systematic re-analysis which will be costly and difficult to arrange (ISPD, 2018).

It is also demonstrated in studies in the USA that parents are keen to receive as much information as possible regarding their child's health, even where results are uncertain (Kalynchuk et al., 2015; Wou et al., 2018). One study used hypothetical scenarios to determine the desire for uncertain results and found that while 86% would like to be told about these results, the proportion decreased as the level of uncertainty increased (Wou et al., 2018). Support systems must be in place which can confidently manage diagnostic uncertainty and explain this adequately to parents. Harris *et al.* describe the counselling and ethical issues which arose in their study of prenatal WES, highlighting how variable these can be and the importance of a case-by-case approach (Harris et al., 2018). Many of these centre around explaining uncertainty to parents in the context of VUS which are potentially pathogenic but lack the required supporting evidence to classify them as such. An approach that minimises any difficulties around reporting is to use a targeted panel to either sequence or interpret WES results. For example, in the PAGE study WES was undertaken but results were interpreted using a targeted virtual gene panel for developmental disorders that included 1628 genes, thus changes in genes such as cancer or autism predisposition but with no prenatal phenotype were not analysed or reported.

Clearly there remains debate over which results should be returned to parents beyond those related to the primary diagnosis. It is likely that there will be considerable variance across the world in these issues but one thing is clear, as suggested in the guidelines published by the ISPD; the nature of the results to be reported must be discussed in detail with parents before

any testing is undertaken, they must be made aware that sequencing could identify findings that may have implications for their own health and they must be allowed time to decide which results they want to receive (ISPD, 2018). In order for adequate informed consent to be obtained parents must be counselled in detail about sequencing, the nature and certainty of the results, the time taken and the fact that interpretation may evolve over time (ISPD, 2018; Quinlan-Jones et al., 2016). They should also receive counselling when the results are available, and this is just as important for those receiving a negative result as a positive one. It seems clear that the counselling burden associated with fetal ES will be large and there is a significant need for health professional education.

The future?

The genetic investigations described above necessitate invasive tests, however, non-invasive prenatal diagnosis (NIPD) is also now available as a diagnostic genetic test for many monogenic disorders (Jenkins et al., 2018), including the use of small panels targeting specific mutations for the diagnosis of conditions caused by mutations in the *FGFR3* gene (Chitty et al., 2015). Cell-free fetal DNA (cffDNA) is already being widely used to detect more common aneuploidies in high risk pregnancies using a variety of technologies (Badeau et al., 2017). Whole genome sequencing of cffDNA has been reported (Kitzman et al., 2012), however, this technology is not yet ready for routine use. Further, analysis of cffDNA is complicated by the high background of maternal cell free DNA as well as DNA from other sources such as in twin pregnancies, or maternal organ transplantation which can result in discrepant results (Hartwig et al., 2017).

Despite these challenges, non-invasive exome sequencing based on fetal cells in the maternal circulation may offer an alternative approach in the future (Chen et al., 2017).

Conclusion

Prenatal WES is proving to be a useful and efficient tool for the rapid investigation of fetal abnormalities, with a promising diagnostic rate considering the 'difficult-to-diagnose' nature of the cohort. With the establishment of NHS Genomic Laboratory Hubs in England, rapid fetal ES has now been commissioned as a clinical diagnostic test within the NHS and in 2019 will be offered for pregnancies complicated by fetal abnormalities with a likely genetic aetiology. Implementation will need to be carefully monitored and the experience used to build on the position statement published by the International Society of Prenatal Diagnosis (ISPD), the Society of Maternal Fetal Medicine (SMFM) and the Perinatal Quality Foundation (PQF) with their 'points for consideration' for laboratories and clinicians offering these services.

Acknowledgments

LSC is an NIHR Senior Investigator and is partially funded by the NIHR Biomedical Research Centre at Great Ormond Street Hospital. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

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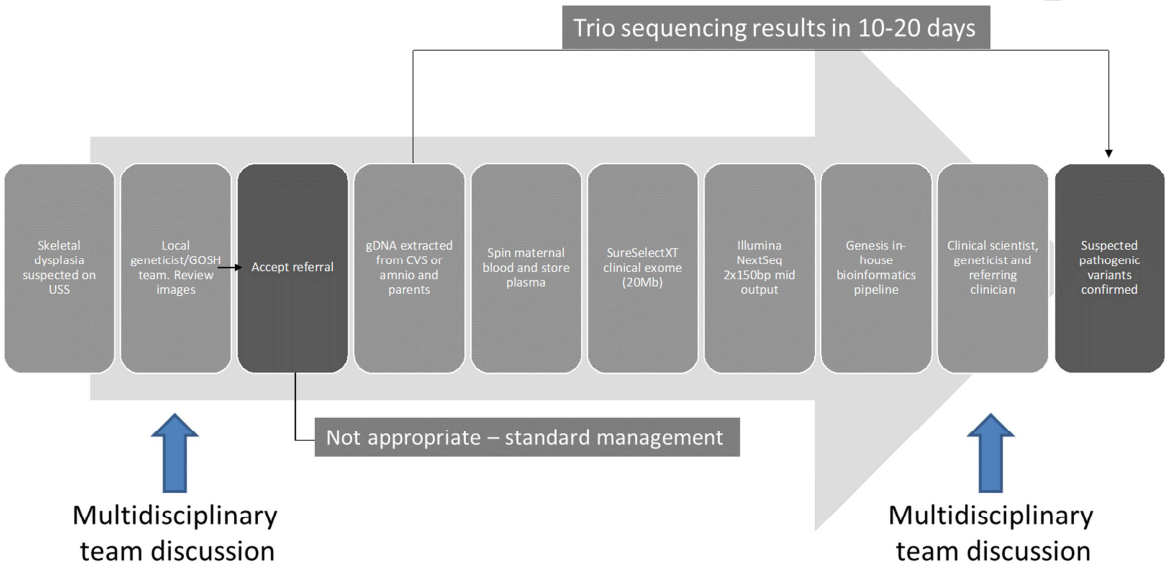
Figure 1 Workflow used for rapid prenatal exome sequencing. Note the multidisciplinary discussion before sequencing to ensure careful case selection, and after sequencing to aid accurate and rapid interpretation of results. Adapted from Chandler *et al.* 2018 with permission.

USS – ultrasound scanning; GOSH – Great Ormond Street Hospital; gDNA – genomic DNA; CVS – chorionic villus sampling; Amnio. – amniocentesis; bp – base pairs



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