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Evolving fetal phenotypes and clinical impact of progressive prenatal exome sequencing pathways: cohort study

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CONTRIBUTION

What are the novel findings of this work?

Prenatal exome sequencing (pES) can give diagnostic information in fetuses with congenital malformations. In this initial cohort, the NHS England R21 prenatal exome sequencing pathway provided a unifying genetic diagnosis in over 50% of pre-selected cases.

What are the clinical implications of this work?

It is important to track fetal phenotype throughout the pregnancy and postnatally when interpreting prenatal exome sequencing results.

ABSTRACT

OBJECTIVES: To determine the; (i) variable diagnostic yield and turnaround time (TAT) of two consecutive prenatal exome sequencing (ES) pathways; (ii) evolution of the fetal phenotype and; (iii) clinical impact in the presence of causative pathogenic variants and incidental findings.

METHODS: This retrospective cohort analysis (of prospectively collected cases) assessed fetuses undergoing trio ES in the presence of structural anomalies with normal chromosome microarray via the West Midlands Regional Genetics Laboratory. This included the periods; (a) 07/2018 to 10/2020 (a post-Prenatal Assessment of Genomes and Exomes (PAGE) pilot study) with prenatal trio ES based on a panel of 1542 development disorder genes and case selection by a multi-disciplinary team and; (b) 10/2020 to 05/2021 with prenatal trio ES based upon the NHS England R21 pathway with definitive inclusion criteria and a panel of 1205 prenatally relevant genes. Deep phenotyping was performed throughout pregnancy and postnatally.

RESULTS: In total n=54 cases were included. The diagnostic yields and TATs for both periods were 28% (n=7/25); 54 (14-213) days and 55.1% (n=16/29) p=0.04; 14.2 (3-29) days respectively. In instances where a causative pathogenic variant was identified, of those reaching the third trimester, additional anomalies were detected between the second and third trimesters in 73.3% (n=11) of cases, predominantly secondary to progressive hydropic features n=3 (27.3%), arthrogryposis n=3 (27.3%) and brain anomalies n=2 (18.2%). In n=3 instances, variants of uncertain significance were upgraded to likely pathogenic based upon postnatal information. Where causative pathogenic variants were detected there was a significant clinical impact in 78.3% (n=18/23), predominantly relating to decision regarding pregnancy course and potential change of neonatal management 38.9% (n=7/18).

CONCLUSION: Prenatal exome sequencing using the NHS England r21 pathway shows great promise when applied to our initial cohort, with a genetic diagnosis obtained in over half of pre-selected ultrasound detected fetal structural anomalies. Tracking and real-time updating of fetal phenotype and consideration of re-classification of variants based upon postnatal findings is vital if one is to optimise the clinical impact already evident from this emergent genomic technology.

INTRODUCTION

Prenatal exome sequencing (ES) has been demonstrated as a highly efficacious diagnostic genetic test in the presence of fetal structural anomalies (FSAs) with a variable diagnostic yield reported of between 20-85%. Optimisation of this yield is dependent upon pre-test genetic selection as well as the anomalous anatomical system and presence of isolated or multiple FSAs.[1-3] Since publication of the Prenatal Assessment of Genomes and Exomes (PAGE) study and similar studies, there is the need and desire to translate ES into routine clinical practice.[4,5] Not only does establishing a prenatal diagnosis limit the diagnostic odyssey many parents endure attempting to obtain a unifying diagnosis, but informs clinical decision making in relation to pregnancy course, delivery, maternal care, neonatal management and mitigation of recurrence using technologies such as preimplantation genetic diagnosis (PGD) and non-invasive prenatal diagnosis (NIPD).[6] In the developed world the translation of ES into clinical practice is taking place, however for this to happen, emerging challenges must be addressed with the support of robust guidance and policy.[7] The interpretation of ES is difficult based upon a prenatal phenotype obtained via ultrasound examination alone due to the limitations in visualisation, phenotypic classification and the fact that the fetus is developing progressively in utero, hence the phenotype may evolve.[8] As a novel testing strategy, there are few studies which display the translation of exclusively prenatal ES from a research setting into clinical practice and to our knowledge none which compare different clinical pathways, hence the aims of this study were to determine the; (i) variable diagnostic yield and turnaround time (TAT) of two consecutive prenatal ES pathways; (ii) evolution of the fetal phenotype and; (iii) clinical impact of detecting a causative pathogenic variants and incidental findings (IFs).

METHODS

Clinical pathways

This retrospective evaluation of prospectively collected cases assessed a cohort of fetuses from April 2019-June 2021 referred to the Birmingham Women's and Children's hospital joint service provided by the West Midlands Fetal Medicine Centre, the Regional Genetics Laboratory and Clinical Genetics service. Inclusion criteria included fetuses with prenatally suspected ultrasound anomalies where chromosome microarray testing was negative and written parental consent was obtained for testing. Trio pES with analysis of a large gene panel was performed based upon prenatal phenotype only, which may have included instances where post-mortem examination was not performed in an instance of perinatal death and a fetal blood sample or fetal tissue was the source of proband DNA. Throughout the study period, clinical pathways evolved;

(i) July 2018-October 2020 – Following completion of the PAGE Study, for which Birmingham Women's Hospital was a primary hub, the clinical study pathway was translated into clinical practice (as a pilot), whilst developing the clinical infrastructure and commissioning for the prenatal genomic service from NHS England was established. This was in keeping with methodology published in the PAGE study with ES performed at the West Midlands Regional Genetics Laboratory.[4] Included patients were those selected prenatally or postnatally dependent upon availability of fetal DNA, with cases with ES testing based upon post-mortem or clinical neonatal examination excluded. All cases were selected based upon multidisciplinary team (MDT) meeting decision involving fetal medicine sub-specialists, clinical geneticists, genomic scientists and midwives.

(ii) October 2020-July 2021 – NHS England commissioning led to delivery of the National Genomic Test Directory [URL: https://www.england.nhs.uk/publication/national-genomic-test-directories/], which included rapid prenatal exome sequencing (R21) via one of two Genomic Laboratory Hubs (GLHs). Our NHS Genomic Medicine Centre fell under the geographical region of the NHS England West Midlands, Oxford and Wessex GLH. Prenatal testing via the rapid prenatal exome sequencing R21 pathway in ongoing pregnancies, with multiple, multisystem, major structural and selected

isolated fetal anomalies was performed centrally in line with a set protocol.[9] Postnatal cases once considered as part of the pre-October 2020 pathway fell under separate pathways of R412 or 'non-urgent congenital malformation and dysmorphism syndromes' and R14 'acutely unwell children with a likely monogenic disorder' which were not included in this study as testing was performed at a different jurisdiction.

Information pertaining to fetal phenotype was collated prospectively throughout pregnancy and postnatally. TAT (days) was taken to mean time in working days from receipt of the sample in the laboratory inclusive of commencement of CMA & culturing to release of the ES report.

Exome sequencing and variant interpretation

Ultrasound confirmation of the suspected FSAs was performed by an accredited fetal medicine specialist with the phenotype described in Human Phenotype Ontology terms.[10] In line with hospital policy, where women consented to first trimester screening for aneuploidy they underwent a first trimester anatomy scan. CMA (via comparative genomic hybridization) of proband DNA was initiated following a negative Quantitative Fluorescent Polymerase Chain result for common aneuploidy and sex chromosome anomalies. In the former part of the study CMA was complete before ES was considered and in the latter, it was performed in parallel with ES analysis (where there was sufficient fetal material), with analysis ceasing should an explanatory CMA result be found. Trio ES was sought in all instances. Pre-October 2020 target enrichment was performed using the Illumina TruSight One Panel, followed by Illumina HiSeq 2500 to sequence the coding and splice site regions in the same 1542 panel of relevant developmental disorder genes as adopted in PAGE with a minimum coverage depth of 20X. After October 2020 an Exome CG target enrichment system (Nonacus) was utilised along with Illumina NextSeq550 assessing coding and splice regions of a panel of 1205 prenatally relevant genes. Variants were filtered in Congenica software using maximum allele frequency >0.01, Variant Effect Predictor (VEP) consequence, PolyPhen score, relevance to phenotype and mode of inheritance. Variants were classified as class I to V in line with guidance from the American College of Medical Genetics and Genomics and The Association for Clinical Genomic Science.[11,12] Variants of uncertain significance (VUS), although discussed,

were only reported in unique circumstances. IFs were minimised by use of a gene panel, and where detected reported on a case-by-case basis as determined by the MDT.

Analysis and approval

Information was gathered in an anonymised fashion included patient demographics, prenatal (and if relevant postnatal) phenotype and ES result in addition to whether there was a significant clinical impact from the ES result (as agreed by F.M. and S.D). The diagnostic yield from different pathways were compared with Chi square testing using IBM SPSS version 27. This study was prospectively approved by the Birmingham Women's and Children's clinical audit department – CARMS-30815 and written consent for inclusion was obtained from patients.

RESULTS

In total n=54 cases underwent ES based upon the prenatal phenotype (pre-R21 n=25 (46.3%) and post-R21 n=29 (53.7%)). The demographic characteristics and outcomes of the study cohort are shown in Table 1. There was a higher than average incidence of South Asian patients and consanguinity due to the underlying demographics of the region, as previously described.[13] All causative pathogenic (class IV and V) variants and IFs with respective outcomes are demonstrated in Table 2, where reported VUS and IFs are displayed in Table 3. Supplementary Table 1 outlines the fetal phenotype where no causative variant was identified.

Comparing pathways

The diagnostic yields from ES from the pre- and post-R21 pathways were 28% (n=7/25) and 55.1% (n=16/29) p=0.04 respectively. Overall, VUS and IFs were reported in 1.9% (n=1/54) and 3.7% (n=2/54) cases respectively [Table 3]. In case ES28 a consanguineous couple had recurrence of the same anomaly with a previous neonatal death (NND). Targeted CMA in the father did not reveal a variant in the SZT2 gene and the finding did not fit with the phenotype, hence was deemed incidental. In ES31 a post-mortem was not performed (as consent was declined) and this case followed two previous NNDs with similar anomalies in a consanguineous couple. This variant was classified as a VUS due to lack of functional studies and clinical data. In the case of ES33 although there were no fetal features of cleidocranial dysplasia the father presented with a mild phenotype of dental anomalies and recurrent ear infections. This was considered an IF with follow-up examination of the baby required postnatally. The mean turnaround times for both pathways were 54.0 (range 14-213) days and 14.2 (range 3-29) days respectively. An initial variant report was obtained antenatally in 40% (n=10/25) and 96.6% (n=28/29) p=0.0001 of cases for both period respectively, with the remaining established postnatally secondary to turnaround time.

Evolution of phenotype

Supplementary Table 2 shows the phenotype in each trimester and the respective postnatal phenotype for all pathogenic variants. In the majority of documented instances, first trimester anatomy was described as normal at 76.2% (n=16/21). Where pregnancies progressed (n=15), more anomalies came to light between the second and third trimesters in 73.3% (n=11) of cases, predominantly secondary to progressive hydropic features n=3 (27.3%), arthrogryposis n=3 (27.3%) and brain anomalies n=2 (18.2%). In one instance hydropic features regressed. There were n=3 (5.6%) cases where variants were upgraded from class III to class IV based upon the availability of information postnatally. In the case of ES39 a PORCN variant was detected but did not fully fit with the phenotype [initially the fetus having a unilateral pleural effusion, a small VSD and foreshortened long bones (on 5th centile for gestation] (and was initially classified as a class III variant), In the late second trimester syndactyl of the feet was noted and post-delivery neonatal erythematous skin patches (focal dermal hypoplasia) developed. This variant then fitted with the phenotype (Goltz-Gorlin syndrome). ES41 & ES42 was the case of a dichorionic twin pregnancy, where initially a homozygous VUS in the PIEZO1 (a new mutation never reported as being associated with NIHF) gene was detected in both fetuses for which the non-consanguineous parents were carriers. Following post-mortem examination the finding of peri-bronchial connective tissue lymphangiectasia in both fetuses and further haematological functional studies via ektacytometry demonstrated the absence of stomatocytes in the parents, hence suggesting healthy carrier status and that this homozygous variant (now upgraded to class IV) represented generalised lymphatic dysplasia in the fetus (autosomal recessive inheritance) as opposed to dehydrated hereditary stomatocytosis (double dominant) for which this variant is also associated. Overall, amongst those cases where a causative pathogenic variant was identified, most had a phenotype affecting more than one anatomical system 71.4% (n=15/21), with the commonest phenotype that of evident or emerging hydrops fetalis; 42.9% (n=9/21) and structural brain anomalies; 42.9% (n=9/21).

Clinical impact

Where a causative pathogenic variant was detected, an significant clinical impact was documented in 78.3% (n=18/23) of cases. This included; (i) guided prenatal counselling with

appropriate specialists e.g. pediatric endocrinology and neonatology n=5 (21.7%); (ii) prompting further history and investigation to unveil a potential previously unknown parental phenotype n=3 (13%) due to autosomal dominant inheritance and partial expression of phenotype (ES2 & ES19) or where carrier status can confer a phenotype in the case of ES37 where maternal carrier status can lead to a cardiomyopathy, prompting cardiac screening; (iii) informed decision making regarding the parental choice of termination pregnancy (TOP) or potential re-direction of neonatal care with palliation n=7 (30.4%) and; (iv) referral for PGD or NIPD n=5 (21.7%).

DISCUSSION

This cohort study demonstrates the rapid evolution from a research-based pathway to a robust and efficient R21 NHS England pathway for performing and reporting of prenatal ES in a clinically relevant timeframe. In instances of causative pathogenic variants, prenatal phenotypes can become more severe and postnatal information may aid in the reclassification of variants. The finding of a causative pathogenic variant by prenatal ES led to a clinical impact in the vast majority.

With the evolution of prenatal CMA, similar to ES, the platforms and interpretational approach used have evolved to provide an improved diagnostic yield, TAT and reduced cost and incidence of incidental findings, as the technologies and clinical pathways have improved.[14-16] In the early stages of prenatal ES, this study shows that such evolution is already evident when a robust and refined clinical pathway and inclusion criteria are used. If one compares the yield from the PAGE study, which included unselected anomalies to more targeted prenatal ES studies where case selection is based upon careful deep phenotypic assessment jointly by a clinical fetal medicine specialist and geneticist; the improved yield is evident.[4,17] In the resource-limited National Health Service where investigations must prove cost-effective, and with such an expensive test, the need for such a targeted selection of cases undergoing prenatal ES has never been more important.[18] In addition to the robust infrastructure offered from the NHS England genomic testing pathway, comes attached governance and auditing to optimise accountability. Even since the initiation of data collection for this study, the R21 pathway has evolved as more knowledge on diagnostic yield for specific phenotypes has been gathered. An example of this has been the addition of hydrops as an indication for offering prenatal ES.[19,20] Refinement of the pathway, to perform CMA in parallel with CMA and re-route postnatal cases to alternative pathways, focuses time and cost into establishing a diagnosis prenatally and thus optimising clinical impact. This highlights the importance of retrospective re-analysis with novel redefined expanded fetal anomaly panels to assess if a pathogenic variant may be uncovered where it had not been previously.[21,22]

It is unsurprising that hydropic phenotypes and brain anomalies provided the greatest yield in the setting of a multisystem disorder.[19] While a meta-analysis of the clinical utility of ES in the setting of neurodevelopmental disorders has been conducted, providing a yield of over 50%, a collation of studies in the setting of prenatal structural brain anomalies is yet to be performed.[23] The reason why deep phenotyping in the fetus is challenging is because imaging does not reveal the subtle dysmorphologies often needed to clinch a unifying genetic diagnosis, with the clinician at the peril of fetal position, maternal habitus and other issues which can make imaging challenging. While the use of 3D-ultrasound to assess for fetal surface rendering dysmorphology has been described, it's accuracy in the setting of prenatal ES has yet to be formally assessed.[24] Further to this, imaging cannot determine function neurodevelopmentally or physiologically, nor does it replace the information provided from a thorough neonatal examination. This is evident from the cases where variants were up-graded following details of the postnatal phenotype. Whether a variant and corresponding phenotype have been described previously through genomic databases confers strong evidence for pathogenicity. As no such databases for fetal phenotype exist, it can be a challenge to confer pathogenicity until postnatal information becomes available.[4] As many fetal phenotypes attributed to pathogenic variants are not seen in neonatal life due to their lethality, this in addition precludes description of associated phenotypes within clinical databases. It is important to assess the fetal phenotype throughout pregnancy when interpreting variants, particularly in brain anomalies where evolving structural differences on imaging are important to be aware of as the fetal brain matures and develops.[25]

With the recognition of several autosomal dominant inherited pathogenic variants in the study, provoking additional history and physical evaluation of the parents, this demonstrates the pitfall of the R21 pathway compared to smaller panel testing and the risk of filtering out heterozygous paternally inherited variants and the need for consideration of this within the bioinfomatic filtering pathway as well as thorough examination and history taking in the parents, particularly where variants can have variable penetrance and their phenotypic impact is not evident.[26] What is probably most important is the clinical impact prenatal ES has on decision making and potential future management of neonates and subsequent pregnancies as well as the impact on the wider family. Its role in this has been supported by

existing studies and will continue to be the main driver for roll-out and advancement of the service within the UK.[27]

The findings of this study are novel in that they are one of the first to attempt to demonstrate the clinical utility of the NHS England R21 pathway for prenatal ES. Further to this, our data collection was almost complete and is one of the first to track fetal phenotype throughout pregnancy and beyond. Limitations include not only the relatively small number of our cohort study, but it's retrospective analysis which limit drawing definitive conclusions. In addition, due to a high incidence of consanguinity within our baseline population, one could question overall generalizability within the UK. For the purposes of our study due to the contingent and then parallel approach to CMA testing and pES adopted, TAT was defined as timing of receipt of laboratory sample for culturing and CMA until release of the ES result. Moving forward for future auditing comparable with TATs for other institutions this should be initiated at the onset of sequencing only. While we did not include the number or outcome of cases where pES was rejected we understand that this will form part of the R21 annual national audit and is an objective of the awaited Optimising Exome PREnatal Sequencing Services (EXPRESS) study.[28] Typical reasons for exclusion evident thus far from provisional R21 findings include; (i) anomalies which don't meet eligibility criteria; (ii) imminent fetal demise or; (iii) alternative panel or single gene testing determined more appropriate.[29]

In conclusion, the R21 NHS England pathway for prenatal ES when applied to our cohort shows great promise with a genetic diagnosis obtained in over half of fetal structural anomalies. Tracking of the fetal phenotype and consideration of re-classification of variants based upon postnatal findings is vital if one is to optimise the clinical impact already evident from this emergent technology.

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CONFLICT OF INTEREST: MDK is a member of Illumina's International Perinatal Advisory Group (but receives no payment for this) and is the Fetal Medicine Representative for the Central and South GLH for which SA is also a member. MDK is also the RCOG representative on the Joint Colleges Committee of Genomic and Genetic Medicine. MDK is funded through the Department of Health, Wellcome Trust and Health Innovation Challenge Fund (award number HICF-R7- 396) for the PAGE and PAGE2 research studies complete August 2019. FM is co-editor for Ultrasound in Obstetrics & Gynecology (Genetics) but has taken no part in peer review or decision with regard article publication. All other authors declare no competing interests.

REFERENCES

1. Han J, Yang YD, He Y, Liu WJ, Zhen L, Pan M, Yang X, Zhang VW, Liao C, Li DZ. Rapid prenatal diagnosis of skeletal dysplasia using medical trio exome sequencing: Benefit for prenatal counseling and pregnancy management. Prenat Diagn. 2020;40(5):577-584.
2. Lei TY, Fu F, Li R, Yu QX, Du K, Zhang WW, Deng Q, Li LS, Wang D, Yang X, Zhen L, Li DZ, Liao C. Whole-exome sequencing in the evaluation of fetal congenital anomalies of the kidney and urinary tract detected by ultrasonography. Prenat Diagn. 2020 ;40(10):1290-1299.
3. Mone F, Eberhardt RY, Morris RK, Hurles ME, McMullan DJ, Maher ER, Lord J, Chitty LS, Giordano JL, Wapner RJ, Kilby MD; CODE Study Collaborators. COngenital heart disease and the Diagnostic yield with Exome sequencing (CODE) study: prospective cohort study and systematic review. Ultrasound Obstet Gynecol. 2021 Jan;57(1):43-51.
4. Lord J, McMullan DJ, Eberhardt RY, Rinck G, Hamilton SJ, Quinlan-Jones E, Prigmore E, Keelagher R, Best SK, Carey GK, Mellis R, Robart S, Berry IR, Chandler KE, Cilliers D, Cresswell L, Edwards SL, Gardiner C, Henderson A, Holden ST, Homfray T, Lester T, Lewis RA, Newbury-Ecob R, Prescott K, Quarrell OW, Ramsden SC, Roberts E, Tapon D, Tooley MJ, Vasudevan PC, Weber AP, Wellesley DG, Westwood P, White H, Parker M, Williams D, Jenkins L, Scott RH, Kilby MD, Chitty LS, Hurles ME, Maher ER; Prenatal Assessment of Genomes and Exomes Consortium. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. Lancet 2019; 393: 747–757.
5. Petrovski S, Aggarwal V, Giordano JL, Stosic M, Wou K, Bier L, Spiegel E, Brennan K, Stong N, Jobanputra V, Ren Z, Zhu X, Mebane C, Nahum O, Wang Q, Kamalakaran S, Malone C, Anyane-Yeboa K, Miller R, Levy B, Goldstein DB, Wapner RJ. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet 2019;393:758-67

6. Tolusso LK, Hazelton P, Wong B, Swarr DT. Beyond diagnostic yield: prenatal exome sequencing results in maternal, neonatal, and familial clinical management changes. Genet Med 2021:13;1-9
7. Mone F, McMullan DJ, Williams D, Chitty LS, Maher ER, Kilby MD; Fetal Genomics Steering Group of the British Society for Genetic Medicine; Royal College of Obstetricians and Gynaecologists. BJOG. 2021;128(9)e39-e50
8. Ferretti L, Mellis R, Chitty LS. Update on the use of exome sequencing in the diagnosis of fetal abnormalities. Eur J Med Genet. 2019;62(8):103663
9. NHS England. Rapid Exome Sequencing Service for Fetal Anomalies Testing. 2021. Accessed from: http://www.labs.gosh.nhs.uk/media/1396328/guidance_document_-_rapid_exome_sequencing_service_for_fetal_anomalies_v3.pdf Accessed on 27th September 2021
10. Köhler S, Gargano M, Matentzoglu N, Carmody LC, Lewis-Smith D, Vasilevsky NA, Danis D, Balagura G, Baynam G, Brower AM, Callahan TJ, Chute CG, Est JL, Galer PD, Ganesan S, Griese M, Haimel M, Pazmandi J, Hanauer M, Harris NL, Hartnett MJ, Hastreiter M, Hauck F, He Y, Jeske T, Kearney H, Kindle G, Klein C, Knoflach K, Krause R, Lagorce D, McMurry JA, Miller JA, Munoz-Torres MC, Peters RL, Rapp CK, Rath AM, Rind SA, Rosenberg AZ, Segal MM, Seidel MG, Smedley D, Talmy T, Thomas Y, Wiafe SA, Xian J, Yüksel Z, Helbig I, Mungall CJ, Haendel MA, Robinson PN. The Human Phenotype Ontology in 2021. Nucleic Acids Research 2021;49(D1):D1207–D1217
11. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17(5):405-24.
12. Ellard S, Baple EL, Callaway A, Berry I, Forrester N, Clare Turnbull4, Martina Owens1, Diana M Eccles8, Stephen Abbs9, Richard Scott4,10, Zandra C Deans11, Tracy Lester12, Jo Campbell13, William G Newman14,15, Simon Ramsden14 and Dominic J McMullan16. ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020. Available from www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf on 24th March 2020

13. Mone F, Doyle S, Ahmad A, Abu Subieh H, Hamilton S, Allen S, MArton T, Williams D, Kilby MD. Diagnostic and perinatal outcomes in consanguineous couples with a structural fetal anomaly: a cohort study. Acta Obstet Gynecol Scand. 2021; 100(3):418-424
14. Robson SC, Chitty LS, Morris S, Verhoef T, Ambler G, Wellesley DG, Graham R, Leader C, Fisher J, Crolla JA. Evaluation of Array Comparative genomic Hybridisation in prenatal diagnosis of fetal anomalies: a multicentre cohort study with cost analysis and assessment of patient, health professional and commissioner preferences for array comparative genomic hybridisation (Southampton (UK), 2017).
15. Chong HP, Hamilton S, Mone F, Cheung KW, Togneri FS, Morris RK, Quinlan-Jones E, Williams D, Allen S, McMullan DJ, Kilby MD. Prenatal chromosomal microarray testing of fetuses with ultrasound structural anomalies: A prospective cohort study of over 1000 consecutive cases. Prenat Diagn. 2019 ;39(12):1064-1069.
16. Levy B, Burnside RD. Are all chromosome microarrays the same? What clinicians need to know. Prenat Diagn. 2019;39:157-64
17. Normand EA, Braxton A, Nassef S, Ward PA, Vetrini F, He W, Patel V, Qu C, Westerfield LE, Stover S, Dharmadhikari AV, Muzny DM, Gibbs RA, Dai H, Meng L, Wang X, Xiao R, Liu P, Bi W, Xia F, Walkiewicz M, Van den Veyver IB, Eng CM, Yang Y. Clinical exome sequencing for fetuses with ultrasound abnormalities and a suspected Mendelian disorder. Genome Med. 2018 Sep 28;10(1):74
18. Kodabuckus SS, Quinlan-Jones E, McMullan DJ, Maher ER, Hurles ME, Barton PM, Kilby MD. Exome Sequencing for Prenatal Detection of Genetic Abnormalities in Fetal Ultrasound Anomalies: An Economic Evaluation. Fetal Diagn Ther 2020;47(7):554-564.
19. Mone F, Eberhardt RY, Hurles ME, McMullan DJ, Maher ER, Lord J, Chitty LS, Dempsey E, Homfray T, Giordano JL, Wapner RJ, Sun L, Sparks TN, Norton ME, Kilby MD.Fetal hydrops and the Incremental yield of Next generation sequencing over standard prenatal Diagnostic testing (FIND) study: prospective cohort study and meta-analysis. Ultrasound Obstet Gynecol. 2021 Apr 13. doi: 10.1002/uog.23652. Online ahead of print.
20. Mellis R, Eberhardt RY, Hamilton SJ; PAGE Consortium, McMullan DJ, Kilby MD, Maher ER, Hurles ME, Giordano JL, Aggarwal V, Goldstein DB, Wapner RJ, Chitty LS. Fetal

exome sequencing for isolated increased nuchal translucency: should we be doing it? BJOG. 2021 Aug 19. doi: 10.1111/1471-0528.16869. Online ahead of print.

21. Costain G, Jobling R, Walker S, Reuter MS, Snell M, Bowdin S, Cohn RD, Dupuis L, Hewson S, Mercimek-Andrews S, Shuman C, Sondheimer N, Weksberg R, Yoon G, Meyn MS, Stavropoulos DJ, Scherer SW, Mendoza-Londono R, Marshall CR. Periodic reanalysis of whole-genome sequencing data enhances the diagnostic advantage over standard clinical genetic testing. Eur J Hum Genet. 2018 ;26(5):740-744.
22. Salfati EL, Spencer EG, Topol SE, Muse ED, Rueda JR, Wagner GN, Campman S, Topol EJ, Torkamani A. Re-analysis of whole-exome sequencing data uncovers novel diagnostic variants and improves molecular diagnostic yields for sudden death and idiopathic diseases. Genome Medicine 2019;111:83
23. Srivastava S, Love-Nichols JA, Dies KA, Ledbetter DH, Martin CL, Chung WK, Firth HV, Frazier T, Hansen RL, Prock L, Brunner H, Hoang N, Scherer SW, Sahin M, Miller DT, NDD Exome Scoping Review Work Group. Meta-analysis and multidisciplinary consensus statement: exome sequencing is a first-tier clinical diagnostic test for individuals with neurodevelopmental disorders
Genet Med. 2019 Nov;21(11):2413-2421.
24. Dall'Asta A, Schievano S, Bruse JL, Paramasivam G, Kaihura CT, Dunaway D, Lees CC. Quantitative analysis of fetal facial morphology using 3D ultrasound and statistical shape modeling: a feasibility study. Am J Obstet Gynecol 2017 Jul;217(1):76.e1-76.e8.
25. Girard N, Chaumoitre K, SConfort-Gouny S, Viola A, Levrier O. Magnetic resonance imaging and the detection of fetal brain anomalies, injury, and physiologic adaptations. Curr Opin Obstet Gynecol 2006 Apr;18(2):164-76.
26. Requena, T., Gallego-Martinez, A. & Lopez-Escamez, J.A. A pipeline combining multiple strategies for prioritizing heterozygous variants for the identification of candidate genes in exome datasets. *Hum Genomics* 11, 11 (2017).
27. Dempsey E, Haworth A, Ive L, Dubis R, Savage H, Serra E, Kenny J, Elmslie F, Greco E, Thilaganathan B, Mansour S, Homfray T, Drury S. A report on the impact of rapid prenatal exome sequencing on the clinical management of 52 ongoing pregnancies: a retrospective review. BJOG. 2021 May;128(6):1012-1019.
28. Chitty L, McEwan A, Tapon D, Ledger J, Kroese M, Hill M, McMullan D, Fisher J, Leeson-Beevers K, Knight M, Parker M, Fulop N, Ellard S, Morris S. Optimising Exome Prenatal

Sequencing Services (EXPRESS). NIHR funding and awards. Accessed from: fundingawards.nihr.ac.uk/award/NIHR127829 on 30th November 2021

29. Chandler N, Allen A, McMullan D, Marks L, Deans Z, Kilby M, Mellis R, Chitty L. P-158 Implementing a national fetal sequencing service for the rapid diagnosis of monogenic conditions in pregnancies complicated by fetal abnormalities: Early lessons learnt. Prenat Diagn. 2021; 41(S1):10-153

Table 1 – Demographics and outcomes in the entire cohort [ES=exome sequencing]

Variable	Mean (+/-SD) or N (%) N=54
Maternal age (yrs)	31 (+/-5.7)
Parity	1 (+/-2.3)
Previous pregnancy losses	2 (+/-1.1)
Consanguinity	11 (20.4)
Ethnicity	
Caucasian	30 (55.6)
South Asian	18 (33.3)
Black African	2 (3.7)
Middle Eastern	4 (7.4)
Gestation at testing (wks)	23.3 (6.1)
Source of fetal DNA	
Amniocytes	31 (57.4)
Chorionic villi	9 (16.7)
Fetal blood/tissue	10 (18.5)
Pleural/pericardial fluid	4 (7.4)
Trio analysis	53 (98.1)
Turnaround time for ES	33.1 (+/-43.1)
Pregnancy outcome	
Livebirth	26 (48.1)
Intrauterine death	5 (9.3)
Termination of pregnancy	18 (33.3)
Neonatal death	5 (9.3)

Table 2- Pathogenic and likely pathogenic exome sequencing findings based upon prenatal phenotype

No.	Prenatal phenotype	Variant	Class	Disease	Outcome
ES2	Bilateral pleural effusions	RASA1 (NM_002890.2): c.2603+2T>A het mat	IV	Capillary malformation-arteriovenous malformation 1	Livebirth
ES3	Cystic hygroma, ventriculomegaly, bilateral talipes, cerebellar anomaly, pleural effusion	KIAA1109 (NM_015312.3): c.3296T>A p.(Leu1099*) & c.12406_12409delTCAG p.(Ser4136Thrfs*2) comp het	IV/V	Alkuraya-Kucinskas syndrome	TOP
ES7	Fixed flexion deformities all four limbs, FGR	ECEL1 (NM_004826.2): c.2055+1G>A & c.494T>C p.(Leu165Pro) c**omp het**	IV/IV	Arthrogrypsosis, distal, type 5D	Livebirth
ES10	Bilateral nephromegaly	BBS10 (NM_024685.3): c.271dupT p.(Cys91Leufs*5) hom	V	Bardet-Biedl syndrome 10	TOP
ES14	Strawberry shaped skull, bowed short long bones with multiple fractures, narrow small thorax	COL1A1 (NM_000088.3): c.1769G>A (p.Gly590Glu) het mat (mosaic)	IV	Osteogenesis imperfecta	TOP
ES16	Polyhydramnios, small stomach, partial ACC, mega cisterna magna, clenched hands overlapping fingers, outlet VSD with aortic override, skin oedema, biventricular hypertrophy	HRAS (NM_005343.4): c.35G>A p.(Gly12Asp), het dn	V	Costello syndrome	NND

ES19	Hydrops fetalis	KRAS (NM_004985.3): c.20T>G p.(Val7Gly), het pat (mosaic)	IV	Noonan syndrome 3	NND
ES20*	VSD, DORV, stomach not visualised, polyhydramnios, FGR, inferior vermian hypoplasia, short long bones	CHD7 (LRG_176t1): c.2735dupC p.(Tyr913Leufs*2) het dn	V	CHARGE syndrome	Livebirth
ES28†	Dysgenesis of corpus callosum, lobar holoprosencephaly	SZT2 (NM_0015284) c.6111C>G (p.Tyr2037Ter) mat	IV	Early infantile epileptic encephalopathy	NND
ES32*	Absent cerebellar vermis, round head, COA, echogenic duplex kidneys, short long bones, bilateral talipes, polyhydramnios, short ribs/narrow chest, FGR	ALG3 (NM_005787.5): c.264DELT p.(Tyr88*) **hom**	V	Congenital disorder of glycosylation	Stillbirth
ES33†	Right-sided atrio-ventricular dilatation, biventricular hypertrophy pericardial and pleural effusion	RUNX2 (NM_001015051.3) c.90dupC p.(Ser31Leufs*130) pat	V	Cleidocranial dysplasia	Livebirth
ES34*	Prenatal oedema, absent nasal bone, increased NF migrognathia, ectrodactyly, bifid hand, absent radius, echogenic kidneys, hypospadias and undescended testes	NIPBL (NM_133433.3): c.6449T>G p.(Leu2150Arg), het dn	V	Cornelia de Lange syndrome	Livebirth
ES37*	Wrists fixed flexed,	TTN (LRG_391t1):	IV	Congenital	Livebirth

	unilateral talipes	c.10303+2T>C p.?&c.38660del p.(Lys12887Argfs*60) comp het		titinopathy	
ES40*	Probable craniosynostosis, bilateral SVC, proptosis, hypertelorism, coloboma	CHD7 (LRG_176t1):c.7701dup p.(Arg2568Thrfs*8) dn	V	CHARGE syndrome	TOP
ES43*	Cystic hygroma, pleural effusion, holoprosencephaly, flexed hands, kyphoscoliosis, banana cerebellum, bilateral talipes, echogenic kidneys	KIAA1109 (NM_015312.3): c.14497C>T p.(Gln4833*) hom	V	Alkuraya-Kucinskas syndrome	TOP
ES46*	Cystic hygroma, ACC, vermian hypoplasia, delayed sulcation	ACTB (LRG_132): c.322A>G p.(Asn111Ser) dn	IV	Baraitser-Winter Cerebro-frontofacial syndrome	TOP
ES47*	NIHF	KMT2D (NM_003482.3): c.14648delG; p.(Ser4883Thrfs*112) dn	V	Kabuki syndrome	Livebirth
ES48*	NIHF, FGR, sloping forehead, ventriculomegaly, oligohydramnios	NBN (LRG_158): c.657_661del; p.(Lys219Asnfs*16) hom	V	Nijmegan Breakage Syndrome	TOP
ES49*	Kyphoscoliosis, hypomineralised round skull, hypotelorism, short ribs, narrow thorax, short proximal	COL1A1 (LRG_1t1): c.2397+2T>C p.(?) het dn	IV	Osteogenesis Imperfecta	TOP

	long bones, distal long bones angulated with single bone, polydactyly, hyperextended feet, gastroschisis				
ES51*	Univentricular heart; DILV, abdominal situs inversus, bilateral ventriculomegaly moderate, right sided stomach	DNAH5 (NM_001369.2): c.6508dupA p.(arg2170Lysfs*23) and c.13285C>T p.(Arg4429*) comp het u/k and mat	V	Primary Ciliary Dysplasia 3	Livebirth
ES53*	Micromelia, fetal growth restriction, bilateral talipes	ARTX (LRG_1153) c.520T>C, p.(Cys174Arg) hemi mat	IV	Alpha-thalassemia X-linked intellectual disability	Livebirth
ES54*	Progressive severe polyhydramnios and reduced fetal movement	MAGED2 (NM_014599.4): c.908C>A p.Ser303*hemi mat	V	Bartter Syndrome, Type 5, antenatal, transient mat	Livebirth

[ACC, agenesis of the corpus callosum; COA, coarctation of the aorta; Comp het, compound heterozygote; dn, *de novo;* DILV, double inlet left ventricle; DORV, double outlet right ventricle; FGR, fetal growth restriction; Hemi, hemizygous; Het, hetererozygous; hom, homozygous; mat, maternally inherited; NF, nuchal fold; NIHF, nonimmune hydrops fetalis; NND ,neonatal death; pat, paternally inherited; SVC, superior vena cava; TOP, termination of pregnancy; u/k, unknown; VSD, ventricular-septal defect] Bold font – consanguineous *R21 pathway † incidental finding

Table 3 - Reported variant of uncertain significance

No.	Prenatal phenotype	Variant	Class	Disease	Outcome
ES31	Right CDH, Dandy-Walker variant, rocker-bottom feet, ambiguous genitalia	COG6 (NM_001145079.1) c.785A>G p.(Tyr262Cys) hom	III	Congenital disorders of glycosylation	NND
ES39*	Large right sided pleural effusion, submembraneous VSD, FGR, syndactyly 2nd-5th feet, short straight long bones <1st centile	PORCN (NM_203475.1): c.373G>A p.(Gly125Arg) hemi dn	IV	Focal dermal hypoplasia	Livebirth
ES41/42*	NIHF in both DCDA twins (dizygotic)	PIEZO1 (LRG_1137t1):c.6905G>C p.(Arg2302Pro) hom	IV	PIEZO1-related generalised lymphatic dysplasia	IUD x 2

[CDH, congenital diaphragmatic hernia; DCDA, dichorionic diamniotic; dn; de novo; FGR, fetal growth restriction; hemi, hemizygous; hom, homozygous; IUD, intrauterine death; VSD, ventricular septal defect] *variant re-classified and up-graded retrospectively

No.	Prenatal phenotype	Outcome
ES1	Exomphalos, bulky 'jelly-like' placenta	NND
ES4	Bilateral enlarged kidneys, anhydramnios, thoracic hypoplasia, suspected omphalocele	TOP
ES5	Hydrops fetalis	Stillbirth
ES6	Increased nuchal translucency, tricuspid atresia, pulmonary stenosis	TOP
ES8	Cystic hygroma, cardiomegaly, phocomelia (legs) & amelia (arms), severe generalised skin oedema, thoracic hypoplasia, suspected coarctation of the aorta, polyhydramnios, absent stomach bubble	NND
ES9	Callosal agenesis, cortical dysplasia, structurally abnormal left frontal lobe, asymmetry posterior fossa structures	Livebirth
ES11	Bilateral radial ray anomalies, kyphoscoliosis, echogenic dysplastic kidney, single femurs, absent tibia/fibula unilaterally, biventricular hypertrophy, cardiomegaly, pericardial effusion, no visible bladder, indeterminate gender, anhydramnios	TOP
ES12	Absent tibia & fibula bilaterally, short femurs, short radii, Tetralogy of Fallot, anal atresia, hypoplastic dysplastic kidneys, oligohydramnios, pelvis/lower spine malformed, caudal regression	TOP
ES13	Ascites, pericardial effusion, mild fetal anaemia (10.4g/dL), polyhydramnios	Livebirth
ES15	Abnormal skull shape, long bones <5th centile, ventricular-septal defect, lumbar hemivertebrae, bilateral talipes	Livebirth
ES17	Pericardial effusion, suspected atrial appendage, bilateral pyelectasis (mild), fetal growth restriction, oligohydramnios	Livebirth
ES18	Isolated ascites	Livebirth
ES21*	Short long bones, talipes	Livebirth
ES22*	Pontocerebellar hypoplasia, fetal growth restriction	TOP
ES23*	Cerebellar hypoplasia, delay in sulcation/gyration and ventriculomegaly	Livebirth
ES24*	Pleural effusion, left sided congenital pulmonary airway malformation	Livebirth

ES25*	Suspected dysgenesis corpus callosum, foreshortened long bones	Livebirth
ES26*	Hydrops fetalis, thoracic hypoplasia, cerebellar/pontine/brainstem hypoplasia, ventriculomegaly, bilateral talipes and extended lower limbs, fixed arms and clenched hands, suspected fetal akinesia	TOP
ES27*	Dysgenesis corpus callosum, right parietal cortical anomaly, interhemispheric cyst	TOP
ES29*	Lemon shaped head, hydrocephalus, lower limb asymmetry, cerebellar hypoplasia, complex congenital heart defect	TOP
ES30*	Polyhydramnios, bilateral talipes, abnormal position of hands, abnormal facial profile - prominent orbits and small chin, suspected fetal akinesia sequence	Stillbirth
ES35*	Increased nuchal translucency, right-transcerebellar arachnoid cyst (Initially suspected cerebellar hypoplasia), semi-membranous ventricular-septal defect	Livebirth
ES36*	Severe unilateral ventriculomegaly	TOP
ES38*	Persistently elevated nuchal fold, left cystic kidney, fetal growth restriction pericardial effusion, short long bones, prominent ventricles	Livebirth
ES44*	Unilateral cleft lip and palate, semi-lobar holoprosencephaly and callosal dysgenesis	Livebirth
ES45*	Ascites and pericardial effusion (resolving), polyhydramnios	Livebirth
ES50*	Duodenal atresia, polyhydramnios, complex heart anomaly	Unknown
ES52*	Raised nuchal fold, tricuspid atresia, ventricular-atrial discordance, severe pulmonary stenosis/atresia	Unknown

Supplementary Table 1 – Prenatal phenotypes and clinical outcomes of cases with a negative exome sequencing result [NND, neonatal death; TOP, termination of pregnancy]

No.	Phenotype				Disease
	First trimester	Second trimester	Third trimester	Postnatal	
ES2	Normal	Small right hydrothorax, echogenic bowel	Significant bilateral pleural effusions	Livebirth at 28-wks. Poor condition. Pleural effusion (one side shunted), anaemia of prematurity, PPHN, CLD, calcified thrombus in IVC. Non-dysmorphic	Capillary malformation-arteriovenous malformation 1
ES3	NT 9.4mm	Cystic hygroma, ventriculomegaly, bilateral talipes, cerebellar anomaly, unilateral pleural effusion	N/A	TOP at 18-wks. No postmortem	Alkuraya-Kucinskas syndrome
ES7	Normal	Bilateral talipes Mild pedal oedema	Fixed flexion deformities all limbs, FGR	Delivery at 39+4 in good condition. Arthrogryposis multiplex congenita, bilateral hip dislocation. Non- dysmorphic otherwise	Arthrogrypsosis, distal, type 5D
ES10	Normal	Bilaterally enlarged polycystic kidneys	N/A	TOP at 24+4 wks. Postmortem; Low set ears, small jaw, distended abdomen, narrow chest, post-axial polydactyly, large cystic dysplastic kidneys, borderline lung hypoplasia, accessory spleen	Bardet-Biedl syndrome 10
ES14	Normal	Strawberry shaped skull, bowed short long bones with multiple fractures, narrow small thorax	Nil additional	TOP at 33+1. No postmortem	Osteogenesis imperfecta
ES16	Normal	Polyhydramnios, small stomach, partial ACC, mega cisterna magna, clenched hands overlapping fingers, outlet VSD with aortic override	Addition of biventricular hypertrophy & skin oedema	NND delivered at 30-wks. Postmortem: Addition of prominent forehead, flat nose, low set-ears, short neck. Overlapping $2^{nd}/3^{rd}$ fingers, grade III IVH, bilateral corneal clouding.	Costello syndrome
ES19	Cystic hygroma	NIHF: Ascites, pleural effusions, thickened	N/A	Induction at 26-wks for maternal mirror	Noonan syndrome 3

		NF, HLHS, non-visualisation of stomach		syndrome and NND. No postmortem	
ES20*	Normal	Mega cisterna-magna, short long bones, echogenic bowel, subaortic VSD and DORV, FGR	Nil additional	Delivery at 38-wks 2.8Kg poor condition. Tetralogy of Fallot, micropenis, unilateral choanal atresia, laryngomalacia, bilateral colobomas, truncal hypotonia	CHARGE syndrome
ES32*	Normal	Dandy Walker variant, dilated 3rd ventricle, thoracic hypoplasia, short long bones, COA, VSD, fixed flexed forearms with adducted thumbs, duplex left kidney, bilateral talipes, polyhydramnios, FGR	Addition of skin oedema, progressive ventricular disproportion, progressive cardiac failure	Stillbirth at 34-wks. No postmortem	Congenital disorder of glycosylation
ES34*	Declined	Prenasal oedema, absent nasal bone, increased nuchal fold, micrognathia, left ectrodactyly, absent radius right arm with rudimentary hand, echogenic kidneys, hypospadias, undescended testes, FGR	Addition of peripheral oedema, unilateral pleural effusion, polyhydramnios, poor gyral/sulcal formation	Livebirth at 36+4 wks. Addition of Pierre robin sequence with posterior cleft palate.	Cornelia de Lange syndrome
ES37*	Normal	Unilateral talipes, fixed flexion wrists, limited fetal movement	Limited views - Arms and legs fixed flexed position	Livebirth at 39+1 wks. Limb anomalies, small VSD with trivial tricuspid regurgitation, respiratory distress	Congenital titinopathy
ES39*	Normal	Large right sided pleural effusion, sub-membraneous VSD, FGR, syndactyly 2nd-5th feet, short straight long bones <1st centile	Resolved pleural effusion, FGR and as previous	At home delivery at 37-wks. Treated for hypothermia. Addition of ectodactyly feet, moderate unilateral hydronephrosis and erythematous skin patches	Focal Dermal Hypoplasia
ES40*	Normal	Asymmetrical skull shape	Probable craniosynostosis,	TOP at 33+3 wks. Postmortem: Flat	CHARGE syndrome

			bilateral SVCs, proptosis, hypertelorism, coloboma	nose, wide nasal bridge, bilateral choanal atresia, asymmetrical face, low set ears, flat philtrum, downslanting eyes, periauricular skin tag, large head, persistent left SVC, arrhinencephaly, asymmetry of brain/skull, irregular skull bones, narrow and irregular sutures and ossification	
ES41/42*	Dizygotic twins Normal x 2	NIHF in both fetuses. Growth discordancy	N/A	Twin stillbirth at 23 and 25-wks. Growth discordancy, Bilateral talipes, fetal hydrops, lung hypoplasia, fetal anaemia, thymus atrophy	Generalised lymphatic dysplasia
ES43*	Cystic hygroma	NIHF; pleural effusion, holoprosencephaly, flexed hands, kyphoscoliosis, banana cerebellum, bilateral talipes, echogenic kidneys	N/A	TOP at 14+4. No postmortem	Alkuraya-Kucinskas syndrome
ES46*	NT 6.1mm	Cystic hygroma, ACC, borderline delayed sulcation, hypertelorism	Inferior vermian hypoplasia, abnormal sulcation most severe in frontal lobes	TOP AT 28+2 wks. Post-mortem; Ventriculomegaly, corpus callosum agenesis, delayed sulcation and vermis abnormality. Secundum-type atrial septal defect. Nuchal oedema.	Baraitser-Winter Cerebro-frontofacial syndrome
ES47*	Missed first trimester screening	Echogenic bowel	NIHF - Ascites, skin oedema, hydrocele. Polyhydramnios	Liverbirth at 39-wks. ASD, reflux and diarrhoea, borderline hypothyroidism. Under investigation for potential seizure activity	Kabuki syndrome

ES48*	Normal	NIHF; hydrothorax, ascites. FGR, sloping forehead, ventriculomegaly, oligohydramnios	N/A	TOP at 28+2 wks. Microcephaly, upslanting eyes, long nose, severe FGR, small internal organs, ectopic horseshoe kidneys	Nijmegan Breakage Syndrome
ES49*	Kyphoscoliosis, hypomineralised round skull, short ribs, narrow thorax, short proximal long bones, distal long bones angulated with single bone, polydactyly, hyperextended feet, anterior abdominal wall defect	N/A	N/A	Surgical TOP at 14+3 wks. Limited postmortem: short irregular upper limb – five fingers Significantly shorter irregularly shaped lower limbs shaped with bony spikes. Five toes. X-rays showed hypomineralised bones with multiple fractures.	Osteogenesis imperfecta
ES51*	Normal	Univentricular heart; DILV, atrial situs inversus, bilateral ventriculomegaly 12mm, right sided stomach	Nil additional	Unknown	Primary Ciliary Dysplasia 3
ES53*	Normal	ECF, unilateral talipes	Bilateral talipes, micromelia with femurs and humeri < 3rd centile. Cerebral redistribution on fetal Doppler.	Livebirth at 37+5 requiring intubation. ASD, PDA, septal hypertrophy, secondary cleft palate, bilateral undescended testes, hypospadias, , bilateral talipes, fetal growth restriction, hypertelorism, low-set rotated ears, large nuchal fold, coagulopathy & sepsis	
ES54*	Normal	Normal	Progressive severe polyhydramnios and reduced fetal movement	Liveborn at 35-wks. No additional findings	Bartter Syndrome, type 5, antenatal, transient

Supplementary Table 2 - Pathogenic and likely pathogenic exome sequencing findings with evolving phenotypes. [ACC, agenesis of the corpus callosum; ASD, atrial septal defect; CLD, chronic lung disease; COA, coarctation of the aorta; DILV, Double inlet left ventricle; DORV, double outlet right ventricle; ECF, echogenic cardiac focus; FGR, fetal growth restriction; HLHS, hypoplastic left heart syndrome; IVC, inferior vena cava; IVH, Intraventricular haemorrhage; N/A, non-applicable; NF, nuchal fold; NIHF, nonimmune hydrops fetalis; NT, nuchal translucency; PDA, patent ductus arteriosus; PPHN, persistent pulmonary hypertension of the newborn; SVC, superior vena cava; TOP, termination of pregnancy; VSD, ventricular-septal defect] *R21 pathway