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Prenatal exome sequencing and impact on perinatal outcome: cohort study

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CONTRIBUTION

What are the novel findings of this work?
A quarter of women with a fetus with multiple anomalies will have late termination of pregnancy (TOP) regardless of whether they have prenatal exome sequencing (pES). Significantly more women opt for late TOP following identification of a causative variant by pES compared with when the result is uninformative, and identification of a causative variant is associated with a longer turnaround time and later TOP.

What are the clinical implications of this work?
The higher incidence of late TOP associated with identification of a causative variant by pES means that earlier screening for fetal anomalies is vital. As the UK National Health Service Rapid Exome Sequencing Service for fetal anomalies testing (R21 pathway) continues to develop, access to pES and turnaround should be optimized, as these factors impact decision timelines. Robust clinical guidance for late TOP and support for couples should be prioritized.

ABSTRACT

Objectives *First, to determine the uptake of prenatal exome sequencing (pES) and the diagnostic yield of pathogenic (causative) variants in a UK tertiary fetal medicine unit following the introduction of the NHS England Rapid Exome Sequencing Service for fetal anomalies testing (R21 pathway). Second, to identify how the decision to proceed with pES and identification of a causative variant affect perinatal outcomes, specifically late termination of pregnancy (TOP) at or beyond 22 weeks' gestation.*

Methods *This was a retrospective cohort study of anomalous fetuses referred to the Liverpool Women's Hospital Fetal Medicine Unit between 1 March 2021 and 28 February 2022. pES was performed as part of the R21 pathway. Trio exome sequencing was performed using an Illumina next-generation sequencing platform assessing coding and splice regions of a panel of 974 prenatally relevant genes and 231 expert reviewed genes. Data on demographics, phenotype, pES result and perinatal outcome were extracted and compared. Descriptive statistics and the* χ*-square or Fisher's exact test were performed using IBM SPSS version 28.0.1.0.*

Results *In total, 72 cases were identified and two-thirds of eligible women (*n = *48) consented to trio pES. pES was not feasible in one case owing to a low DNA yield and, therefore, was performed in 47 cases. In one-third of cases (*n = *24), pES was not proposed or agreed. In 58.3% (14/24) of these cases, this was because invasive testing was declined and, in 41.7% (10/24) of cases, women opted for testing and underwent chromosomal microarray analysis only. The diagnostic yield of pES was 23.4% (11/47). There was no overall difference in the proportion of women who decided to have late TOP in the group in which pES was agreed compared with the group in which pES was not proposed or agreed (25.0% (12/48)* vs *25.0% (6/24);* P = *1.0). However, the decision*

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to have late TOP was significantly more frequent when a causative variant was detected compared with when pES was uninformative (63.6% (7/11) vs *13.9% (5/36);* $P < 0.0009$*). The median turnaround time for results was longer in cases in which a causative variant was identified than in those in which pES was uninformative (22 days (interquartile range (IQR), 19–34) days* vs *14 days (IQR, 10–15 days);* $P < 0.0001$*).*

Conclusions *This study demonstrates the potential impact of identification of a causative variant by pES on decision to have late TOP. As the R21 pathway continues to evolve, we urge clinicians and policymakers to consider introducing earlier screening for anomalies, developing robust guidance for late TOP and ensuring optimized support for couples. © 2022 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Approximately 3.5% of pregnancies have significant fetal structural anomalies. Rapid prenatal exome sequencing (pES) has been an important addition to prenatal diagnosis in the UK and was rolled out in October 2020 within the National Health Service England Rapid Exome Sequencing Service for fetal anomalies testing (R21 pathway)[1,2]. pES is performed for an agreed panel of genes known to cause disorders that present prenatally, after discussion with clinical geneticists. Rapid aneuploidy testing via quantitative fluorescence polymerase chain reaction (QF-PCR) is performed first, and those with no diagnosis proceed to pES, with chromosomal microarray analysis (CMA) and pES done in parallel, if indicated[2]. This service can provide parents with a definitive genetic diagnosis and information with which to evaluate options for the pregnancy, plan neonatal care and consider the risk of recurrence.

Within the UK, fetal anomaly screening is performed during a mid-trimester scan at 18–21 weeks' gestation. This is the time period when most anomalies are detected and referred to a fetal medicine unit, in which pES may be considered[3]. No gestational age limits are applied to termination of pregnancy (TOP) for fetal abnormality. However, there must be consensus from two clinicians that the grounds for Clause E of the Abortion Act are met, i.e. that 'there is a substantial risk that the child, if born, would suffer physical or mental abnormalities that would result in serious handicap'[4]. From 22 weeks' gestation, feticide is recommended to prevent inadvertent live birth[5]. While some studies have detailed the diagnostic yield and relevant phenotypes in pES, the potential impact on perinatal outcome, specifically a decision to have late TOP, has not been addressed.

We aimed to determine the uptake of pES and diagnostic yield of causative variants identified in a UK tertiary fetal medicine center following the introduction of the R21 pathway. By comparing cases in which R21 criteria for pES were fulfilled and pES was agreed with those in which the criteria were met but pES was not proposed or agreed, we aimed to assess how the decision for pES affected pregnancy outcome, specifically late TOP (from 22 weeks' gestation). We also aimed to assess how pES results affected pregnancy outcome, specifically late TOP, by comparing cases in which a causative variant was identified with those in which pES was uninformative.

METHODS

This was a retrospective evaluation of a cohort of anomalous fetuses referred to the Liverpool Women's Hospital Fetal Medicine Unit between 1 March 2021 and 28 February 2022. Approval for this work was obtained from the Liverpool Women's Hospital Quality and Governance Department (SE/0001 Refers approved 01/03/22). All cases underwent testing via the R21 pathway for rapid fetal exome sequencing, primarily within the Central and South Genomic Laboratory Hub (GLH) at the Birmingham Women's and Children's NHS Trust, as per inclusion and exclusion criteria for the R21 pathway (Table S1). Written parental consent was obtained for testing. Further specific parental consent was obtained for discussion of individual cases and images presented in this manuscript (Appendices S1 and S2).

Trio exome sequencing (fetus, mother, father) was performed with a minimum depth of 20X. An Illumina next-generation sequencing platform was used to sequence the whole exome captured by the Nonacus Cell3Target ExomeCG target enrichment (Nonacus, Birmingham, UK). Analysis was carried out on coding and splice regions of a panel of 974 prenatally relevant genes (fetal anomaly gene panel v1.92 PanelApp, green genes only)[6]. In addition, a panel of 231 expert-reviewed genes, for which NHS Genomic Medicine Service approval was pending, was added owing to their association with fetal anomalies (gene list available on request).

Data processing, variant calling and analysis were carried out using clinical decision support platform Congenica™ (Congenica Ltd, Cambridge, UK) against reference human genome GRCh37. Variants were filtered using a maximum population allele frequency of > 0.01, variant effect predictor consequence, relevance to phenotype and mode of inheritance and then classified according to the American College of Medical Genetics and Genomics and the Association for Clinical Genomic Science guidance[7,8]. Variants of uncertain significance (VUS) were not reported unless they were potentially clinically significant. Incidental findings were reported on a case-by-case basis. Turnaround time (TAT) in days was defined as number of working days from receipt of the sample at the central GLH (excluding culture time, DNA extraction, transport and collection of familial samples) until a final report was issued following Sanger validation. Prenatal ultrasound assessment was performed by an accredited fetal medicine specialist with the phenotype described using Human Phenotype Ontology terms[9].

Basic demographic data, phenotype, pES result and perinatal outcome were anonymized before analysis.

Descriptive statistics, the χ-square or Fisher's exact test for categorical variables and *t*-test or Wilcoxon rank-sum test for continuous variables were performed using SPSS version 28.0.1.0 (IBM Corp., Armonk, NY, USA); $P < 0.05$ was considered to indicate statistical significance.

RESULTS

In total, 72 women were found to have fetuses with multiple structural anomalies and/or non-immune hydrops fetalis during the study period (Figure 1). In one-third of cases ($n = 24$), pES was not proposed. In 14/24 (58.3%) cases, this was because the woman declined invasive testing, while in 10/24 (41.7%) cases, women opted for testing and underwent PCR and CMA, but pES was not discussed (Figure 1). In 60.0% (6/10) of cases that underwent CMA without pES, testing was performed in the local unit and women either opted for TOP after tertiary fetal medicine counseling or had an intrauterine death.

In two-thirds of cases ($n = 48$), pES was agreed and women gave consent for trio pES. One discordant dichorionic twin pregnancy was tested. In one case, it was not feasible to proceed with pES owing to a low DNA yield, and a causative variant was subsequently identified by whole genome sequencing following neonatal death. Prenatal ES was completed in 47 cases and the diagnostic yield was 23.4% (11/47). Anomalies in more than one organ system was the indication for pES in 10 of the 11 fetuses; in one case, non-immune hydrops fetalis was the indication for pES. Table 1 shows patient demographics and outcome for the complete cohort, and Table 2 includes all reported causative variants. Tables S2 and S3 include details for all cases in the cohort that underwent TOP and all cases in which pES was uninformative, respectively.

Four of the women whose fetus had a causative variant opted to continue their pregnancy, and there were no neonatal deaths in this group (Cases 20, 21, 43 and 46) (Table 2). In two of these cases, the variant was maternally inherited (Cases 20 and 46). Case 20 presented with pleural effusion and skin edema, which remained stable. The neonate was delivered at 39 weeks. As the monoallelic *EPHB4* variant has variable penetrance, the mother herself was asymptomatic, although follow-up brain imaging was recommended. In Case 46, the mother was thought to have Noonan syndrome clinically and was awaiting whole genome sequencing results, while the fetus was large-for-gestational age and appeared to have accelerated sulcation/gyration and an abnormal cerebellar vermis. The mother opted to continue the pregnancy and delivered at 37 weeks. In Case 21 (Milroy disease), the parents had been trying to conceive for more than 10 years, they were counseled about variable severity of the condition and opted for pleuroamniotic shunting at 30 weeks; labor ensued at 33 weeks. Case 43 (Robinow syndrome) presented with a balanced atrioventricular septal defect and bilateral cleft lip and palate. The parents were counseled that most individuals with this syndrome have good function and quality of life, they were

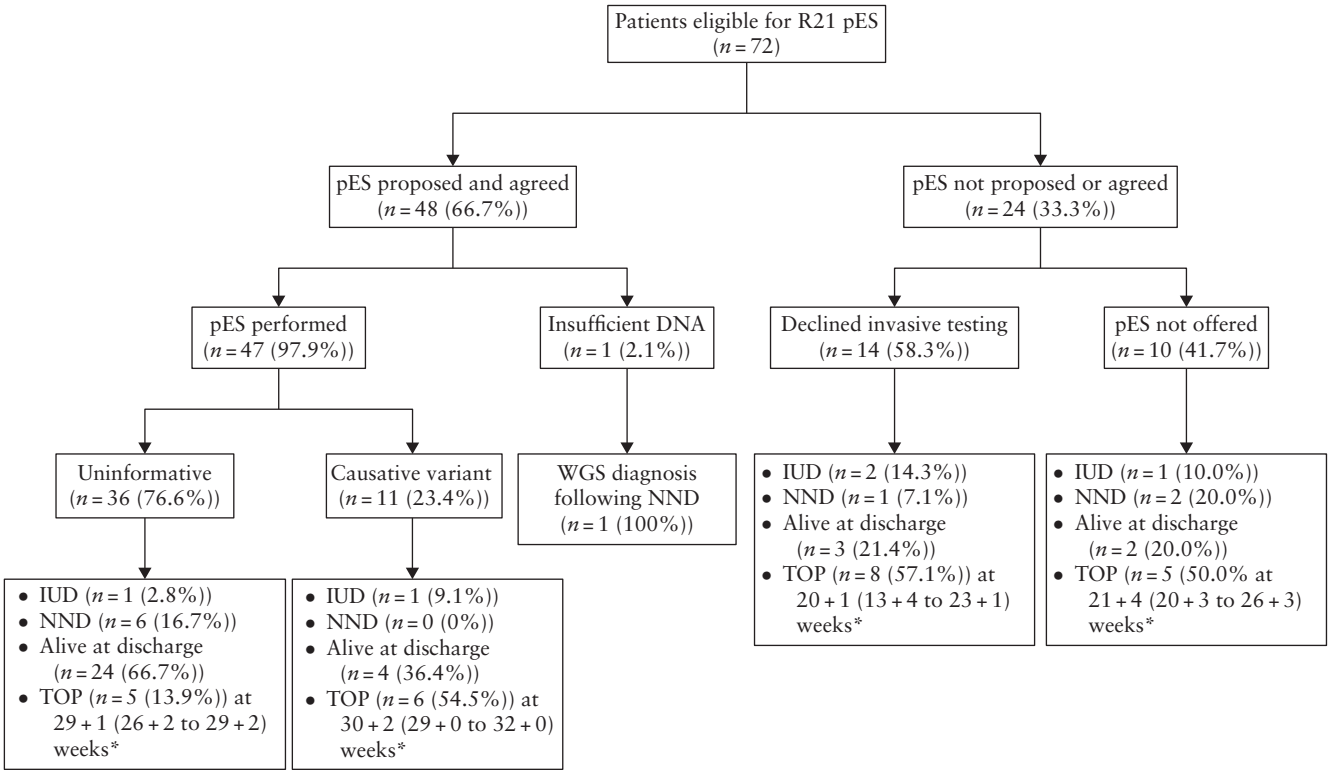

Figure 1 Flowchart summarizing outcomes of patients eligible for prenatal exome sequencing (pES) as per the R21 pathway between March 2021 and February 2022. IUD, intrauterine death; NND, neonatal death; TOP, termination of pregnancy; WGS, whole genome sequencing. *Median (interquartile range).

supported by the pediatric cardiology and cleft teams and delivered at 38 weeks. Additionally, two VUS (Table S4) and one incidental finding (Table S5) were reported.

Overall, there was no significant difference in the proportion of women who decided to have late TOP between the group in which pES was agreed and that in which pES was not proposed or agreed (25.0% (12/48) *vs* 25.0% (6/24); $P = 1.0$). The median gestational age at late TOP was 29 + 2 weeks (interquartile range (IQR), 27 + 6 to 31 + 5 weeks) in the group in which pES was agreed and 24 + 0 weeks (IQR, 23 + 1 to 25 + 0 weeks) in the group in which pES was not proposed or agreed. However, the proportion of women who decided to have late TOP was significantly higher among those with a causative variant on pES than in the group in which pES was uninformative (63.6% (7/11) *vs* 13.9% (5/36); $P < 0.0009$). In one case that decided to have late TOP, this was not performed because intrauterine death occurred. The median gestational age at late TOP was 30 + 2 weeks (IQR, 29 + 0 to 32 + 0 weeks) in cases in which a causative variant was identified by pES and 29 + 1 weeks (IQR, 26 + 2 to 29 + 2 weeks) in which the result was not informative. The median TAT for pES result in the group in which a causative variant was identified was longer than that in the group in which pES was uninformative (22 days (IQR, 19–34 days) *vs* 14 days (IQR, 10–15 days); $P < 0.0001$), which may in part explain the trend toward later decision and completion of TOP in the former group. Two case histories illustrating the impact of a pES result on decision-making are presented in Appendices S1 and S2, while ultrasound findings in the two cases are shown in Figures S1 and S2.

Table 1 Maternal and pregnancy characteristics of study cohort, according to whether women were offered and agreed to prenatal exome sequencing (pES)

Variable	*pES agreed (n = 48)*	*pES not proposed or agreed (n = 24)*
Maternal age (years)	32.5 (26–35)	31.5 (29.5–37.5)
Nulliparous	25 (52.1)	11 (45.8)
Consanguineous	2 (4.2)	0 (0)
Caucasian ethnicity	40 (83.3)	23 (95.8)
Anomaly present		
Central nervous system	19 (39.6)	13 (54.2)
Facial	5 (10.4)	4 (16.7)
Cardiac	14 (29.2)	9 (37.5)
Thoracic	4 (8.3)	3 (12.5)
Gastrointestinal	7 (14.6)	8 (33.3)
Urogenital	15 (31.3)	7 (29.2)
Skeletal	11 (22.9)	8 (33.3)
Non-immune hydrops	7 (14.6)	5 (20.8)
GA at testing* (weeks)	21 + 3 (18 + 6 to 24 + 2)	21 + 5 (16 + 0 to 23 + 4)
Source of fetal DNA		
Amniocytes	46 (95.8)	7/10 (70.0)
Chorionic villi	2 (4.2)	3/10 (30.0)
Turnaround time for pES		
7–14 days	27/47 (57.4)†	—
15–20 days	12/47 (25.5)†	—
≥ 21 days	8/47 (17.0)†	—
Pregnancy outcome		
Alive at discharge from hospital	28 (58.3)	5 (20.8)
Intrauterine death	2 (4.2)	3 (12.5)
Decided to have late TOP (≥ 22 weeks)	12 (25.0)‡	6 (25.0)
TOP performed	11 (22.9)	13 (54.2)
Neonatal death	7 (14.6)	3 (12.5)

Data are given as median (interquartile range), *n* (%) or *n*/*N* (%). *If applicable. †Different denominator used due to insufficient DNA yield in one case. ‡One woman (Case 42) had intrauterine fetal death after she had decided to opt for late termination of pregnancy (TOP). GA, gestational age.

DISCUSSION

In this cohort, pES provided an antenatal genetic diagnosis in almost a quarter of cases. While eligible pregnant women undergoing pES were not more likely to opt for late TOP than were women who did not undergo pES, patients with a causative variant on pES were more likely to choose late TOP than were those in whom pES was uninformative.

The diagnostic yield in our study (23%) is not as high as that reported previously (48–55%)[10,11]. In our cohort, one VUS (Case 12) (Table S4) had the potential for upgrading, which would have increased the yield to 25.5% (12/47). Furthermore, in two cases with an uninformative result on pES, the fetal phenotype regressed or normalized, and by the third trimester, neither of them would have met the criteria for the R21 pathway (Cases 3 and 23) (Table S3). Excluding these cases would increase the yield further to 26.7% (12/45), illustrating both the challenges of analyzing small cohort data and the need for longitudinal fetal phenotyping[12].

There is a paucity of studies reporting on the impact of pES on late TOP, and those published had a small sample size and most did not specify gestational age at TOP. Deden *et al.*[13] reported on 54 cases in which pES was performed and in which outcomes were reported. In their study, the proportion of cases with late TOP in the causative-variant group was greater than that in the group with an uninformative result (40.0% (6/15) *vs* 10.0% (2/20); $P = 0.04$)[13]. De Koning *et al.*[14] also investigated the influence of pES on decision-making regarding late TOP, reporting a trend towards a greater incidence of late TOP in the causative-variant group than in the group with an uninformative result (25.0% (2/8) *vs* 0% (0/12); $P = 0.15$). Mone *et al.*[15] studied 54 cases that underwent pES, reporting a trend towards a greater incidence of late TOP in the causative-variant group than in the group with an uninformative result (30.4% (7/23) *vs* 16.1% (5/31); $P = 0.2$). In the UK, the proportion of TOPs performed for fetal abnormality after 24 weeks has not changed since the introduction of the R21 pathway (0.13% in 2019 *vs* 0.11% in 2020)[5,16,17]. However, these figures do not include all cases that underwent pES as part of the R21 pathway, and it is likely that the total number

of cases with late TOP is too small to reflect a significant change at present. It is important to note that, in the current R21 pathway, pES is not offered to women who have already made a decision to have TOP or who decide to opt for TOP when testing has commenced to prioritize rapid testing for cases in which pES may influence management. In such cases, postmortem examination and discussion at a multidisciplinary fetal dysmorphology meeting are advised to decide on further testing, with a move toward whole genome sequencing.

Table 2 Prenatal phenotype, outcome and exome sequencing findings in cases with a causative variant on prenatal exome sequencing

Case	*Prenatal phenotype*	*Variant*	*ACMG/ACGS classification*	*Disease*	*Outcome*
1	Evolving cortical brain anomaly, bilateral duplex kidneys, microcephaly, small thymus	*SETBP1* (LRG_1150): c.2608G>A p.(Gly870Ser) het dn	V	Schinzel–Giedion syndrome OMIM 269150	TOP
11	Extensive bilateral polymicrogyria, bilateral talipes	*PEX1* (NM_000466.2): c.2916delA p.(Gly973Alafs*16) mat and c.1208delA p.(Asn403Metfs*2) pat	V	Zellweger syndrome OMIM 214100	TOP
14*	Double aortic arch, small thymus, unilateral CLP, brachycephaly	*KAT6A* exon 13 to 17 deletion het dn	V	Arboleda–Tham syndrome OMIM 616268	TOP
16	Microcephaly, increased nuchal fold, unilateral talipes, cerebellar hypoplasia, molar tooth sign, cardiomegaly, renal pelvis dilatation and dysplastic renal cortex, bilateral pyelectasis, oligohydramnios	*PQBP1* (NM_001032381.1): c.155G>A p.(Trp52*) hemi mat	V	Renpenning syndrome OMIM 309500	TOP
20	NIHF	*EPHB4* (NM_004444.4): c.2191C>G p.(Leu731Val) het mat	IV	Capillary malformation arteriovenous malformation syndrome OMIM 618196	CP, LB
21	NIHF, polyhydramnios, dilated right atrium	*FLT4* (NM182925.4): c.3821A>T p.(Asp1274Val) het dn	IV	Milroy disease OMIM 153100	CP, LB
38	Bilateral SVC, bicoronal craniosynostosis, FGR, unilateral pelvic kidney, small other kidney, prefrontal edema	*KMT2D* (NM_003482.3): c.6827del p.(Pro2276Hisfs*10) het dn	V	Kabuki syndrome OMIM 147920	TOP
39	Third-trimester bilateral pleural effusion leading to NIHF, polyhydramnios, unilateral renal pelvis dilatation, bicuspid aortic valve, inlet VSD and aortic stenosis with post-stenotic dilatation of ascending aorta	*PTPN11* (LRG_614t1/NM_002834.3): c.1510A>G p.(Met504Val) het dn	V	Noonan syndrome OMIM 163950	TOP
42	Cystic hygroma, VSD, micrognathia, bilateral talipes, absent corpus callosum, hypoplastic thorax	*KAT6B* (NM-012330.3): c.5238C>A p.(Cys1746*) het dn	V	SBBYS syndrome OMIM 603736	Decided to have TOP but had IUD
43	Bilateral CLP, AVSD	*DVL3* (LRG_1269): c.1745del G p.Gly582Alafs*86 het dn	IV	Robinow syndrome OMIM 616894	CP, LB
46	Polyhydramnios, posterior fossa anomaly, bilateral hydronephrosis, brachycephaly, accelerated sulcation/gyration, maternal historical diagnosis of Noonan syndrome	*CNOT1* (NM_016284.4): c.76C>T p.(Arg26*) het mat	V	CNOT1 neurodevelopmental disorder OMIM 619033	CP, LB

*Sequencing performed via North Thames Genomic Laboratory Hub at Great Ormond Street Hospital for Children NHS Foundation Trust.
ACGS, Association for Clinical Genomic Science; ACMG, American College of Medical Genetics and Genomics; AVSD, atrioventricular septal defect; CLP, cleft lip and palate; CP, continued pregnancy; dn, *de novo*; FGR, fetal growth restriction; hemi, hemizygous; het, heterozygous; IUD, intrauterine death; mat, maternally inherited; LB, live birth; NIHF, non-immune hydrops fetalis; pat, paternally inherited; SBBYS syndrome, Say–Barber–Biesecker–Young–Simpson syndrome; SVC, superior vena cava; TOP, termination of pregnancy; VSD, ventricular septal defect.

In the UK, most cases eligible for pES are detected from 18 weeks onwards, as the fetal anatomy scan is offered between 18 and 21 weeks' gestation. However, earlier assessment of fetal anatomy may benefit the timeline for pES, and earlier identification of a causative variant has the potential to decrease gestational age at TOP in many cases. We identified longer TAT in the group with a causative variant on pES in our cohort, which may be because of the requirement for Sanger validation prior to reporting, as well as the time needed for post-test counseling and decision-making. In cases in which pES is uninformative, the decision to have TOP rests on the significance of structural anomalies present. In our cohort, 72.2% ($n = 26$) of cases in which pES was uninformative were eligible for Clause-E TOP and 19.2% ($n = 5$) of the eligible cases decided to proceed with TOP. Additionally, not all cases eligible for pES or those with a causative variant will be eligible for TOP for fetal abnormality. In our cohort, 81.8% ($n = 9$) of cases in which a causative variant was identified were eligible for Clause-E TOP based on their ultrasound features alone and 66.7% ($n = 6$) of these proceeded with TOP on receipt of their pES results. However, four cases continued their pregnancy (Cases 20, 21, 43 and 46) (Table 2).

The clinical utility of pES is clear, but the R21 pathway is in its infancy. As the R21 pathway evolves, TAT and costs will probably decrease as with previous genomic technologies[18]. As we understand fetal phenotypes more and diagnostic yield with anomalies in different fetal systems increases, the inclusion criteria for R21 will also evolve[19]. Direct communication between clinical and laboratory teams should be encouraged, particularly in instances of evolving phenotypes.

The main strength of our study is that it is a comprehensive analysis of cases eligible for pES following introduction of the R21 pathway. We applied the R21 pathway inclusion criteria, thereby limiting bias in case selection. This is one of the first studies to identify a clinically important increase in the rate of late TOP because of a diagnostic pES result. A relatively small number of cases in our cohort is an obvious weakness; however, owing to the novelty of pES, studies published to date have generally included fewer than 100 cases[20,21]. Data from the planned R21 national audit should provide much larger numbers and complement already available data.

Our cohort reflects application of a UK pathway and abortion law, which limits generalizability of our data to other countries. Moving forward, we must focus on patient education and the importance of a multidisciplinary team, particularly as we note that almost one-fifth of eligible women declined an invasive test and pES was not offered in 14% of eligible cases. Those who are opposed to late TOP should not be denied the opportunity to undergo pES. In countries in which late TOP is legally permissible, seeking an earlier diagnosis with high-quality first- and second-trimester anatomy scans is a priority, along with relevant guidance from clinicians[22].

Conclusions

This study indicates that one in four women that would be eligible for pES choose late TOP after tertiary-level assessment. In cases in which pES is conducted, identification of a causative variant significantly increases the proportion of patients opting for late TOP. As pES inclusion criteria, TAT and access to pES continue to evolve, we urge clinicians and policymakers to consider earlier fetal anomaly screening, longitudinal fetal phenotyping, collaborative work between fetal medicine and clinical genetics teams, and development of resources to support couples.

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Table S1 NHS England rapid fetal exome R21 pathway inclusion and exclusion criteria[1]

Table S2 Ultrasound phenotypes and assessment for Clause E termination of pregnancy based on ultrasound features alone of all cases in which termination of pregnancy was carried out

Table S3 Ultrasound phenotypes, assessment for Clause E termination of pregnancy based on ultrasound features and pregnancy outcome of all cases in which prenatal exome sequencing was uninformative

Table S4 Cases with potentially significant variant of unknown significance on prenatal exome sequencing, absent from population databases and hypomorphic

Table S5 Case with incidental findings on prenatal exome sequencing

Appendix S1 Case 1 history, illustrating the impact of a prenatal exome sequencing result on decision-making

Appendix S2 Case 38 history, illustrating the impact of a prenatal exome sequencing result on decision-making

Figure S1 Ultrasound features identified in a fetus with a *de-novo* heterozygous pathogenic variant in the *SETBP1* gene consistent with Schinzel–Giedion syndrome.

Figure S2 Ultrasound features identified in a fetus with a *de-novo* heterozygous variant in the *KMT2D* gene in keeping with Kabuki syndrome.