

PRENATAL EXOME SEQUENCING AND THE IMPACT ON TERMINATION OF PREGNANCY AFTER 22 WEEKS GESTATION: A COHORT STUDY

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Introduction and Aims

Approximately 3.5% of pregnancies are found to have significant fetal structural anomalies, one in five of these have chromosomal problems detectable by G-banding karyotyping or microarray-based comparative genomic hybridisation (aCGH). More sophisticated genomic testing continues to develop at a significant rate and the introduction of rapid prenatal exome sequencing (pES) has been an important addition to prenatal diagnosis in the UK, rolled out in October 2020 (R21 NHS England Pathway). There are an abundance of studies being released on the diagnostic yield and relevant phenotypes in pES., but the impact on decisions for termination of pregnancy (TOP) is not yet clear.

We aimed to determine the uptake of pES and diagnostic yield of pathogenic and likely pathogenic (causative) variants identified in a UK tertiary Fetal Medicine Centre following the introduction of the R21 NHS England pathway. We also aimed to identify how the decision to proceed with pES and identification of causative variants have affected pregnancy outcomes, specifically late termination of pregnancy (at and beyond 22 weeks gestation).

Methods

This was a retrospective cohort study assessing results and outcomes for anomalous fetuses referred to the Liverpool Women's Hospital Fetal Medicine Unit between 01/03/21 and 28/02/22.

Fetuses with two or more structural anomalies and or non-immune hydrops fetalis were included. All pES were performed as part of the R21 NHS England pathway for rapid fetal exome sequencing. Cases where abnormalities were identified on by Polymerase Chain Reaction (PCR) or aCGH were excluded, as were cases where only postnatal testing took place.

Trio exome sequencing was performed with a minimum depth of 20X using an Illumina NGS platform was used to sequence the whole exome captured by the Nonacus Cell3Target ExomeCG target enrichment. Analysis was carried out on coding and splice regions of a panel of n=974 prenatally relevant genes (Fetal anomaly gene panel v1.92 PanelApp green genes only) and an additional panel of n=231 expert reviewed genes also ascertained due to association with fetal anomaly

Demographics, phenotype and exome sequencing result, and perinatal outcome were extracted and anonymised. Statistical analysis was performed using IBM SPSS version 28.0.1.0. Prospective governance approval was obtained from Liverpool Women's Hospital Quality and Governance Department (approved 01/03/22).

Results

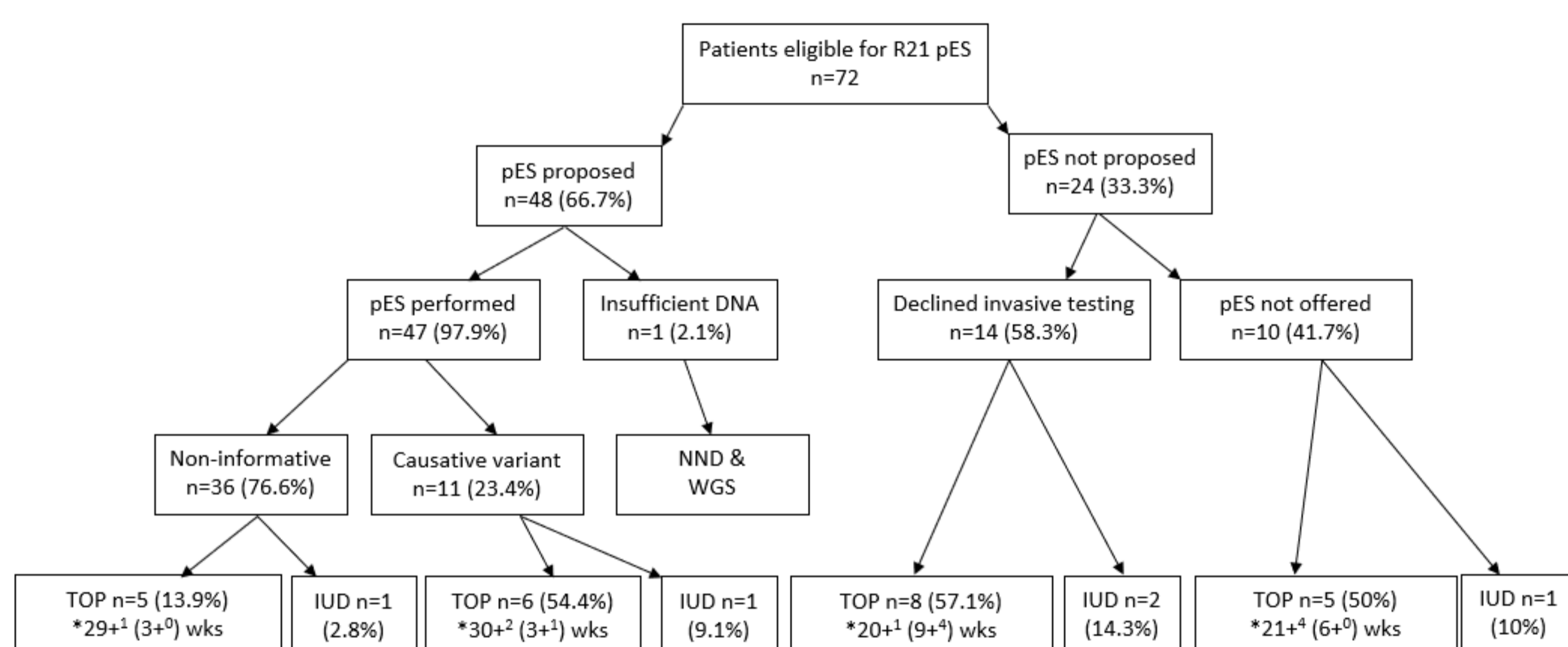


Figure 1. Study flow diagram

- 72 cases were identified during the time period (Figure 1). In two thirds of cases (n=48) pES was agreed and women gave consent for prenatal trio exome sequencing (Figure 1). One discordant dichorionic twin pregnancy was tested. In one case pES was not feasible due to low DNA yield. The final pES group comprised 47 cases (Figure 1).
- In one third of cases (n=24) pES was not proposed (Figure 1). In 58% (n=14) this was because the woman declined invasive testing and in 42% (n=10) women opted for testing and had PCR and aCGH but pES was not discussed (Figure 1). In 60% (n=6) of these cases testing had been commenced in the local unit and women either opted for TOP after tertiary Fetal Medicine counselling or were found to have had an IUFD.
- Where pES was performed, the diagnostic yield of causative variants was 23% (n=11/47, Table 1). There was no significant difference in the proportion of late TOPs between cases where pES was agreed and where pES was not proposed (12/48 25% vs 6/24 25%, p=1.0). The median gestation at late TOP was 29+2 (IQR 3+6) for women after pES and 24+0 (IQR 2+6) for women where pES was not proposed.
- Decisions for late TOP were significantly greater when a causative variant was detected on pES compare to when the pES was non-informative (7/11 63.6% vs.5/36 13.8%, p<0.0009). The median gestation at late TOP was 30+2 (IQR 3+1) in cases where a causative variant was identified by pES and 29+1 (IQR 3+0) where the result was not informative.
- The median turnaround time for results was longer where a causative variant was identified than for those where pES was non-informative (22 days IQR 15 days vs. 14 days IQR 5 days, P=<0.0001).

Prenatal phenotype	Variant	ACMG Classification	Disease	Outcome
Evolving cortical brain anomaly, bilateral duplex kidney, microcephaly, small thymus	SETBP1 (LRG_1150): c.2608G>A p.(Gly870Ser) het <i>dn</i>	V	Schizel-Giedion syndrome	TOP
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	TOP
Double aortic arch, small thymus, unilateral CLP, brachycephaly	KAT6A Exon 13 to 17 deletion het <i>dn</i>	V	Arboleda-Tham syndrome	TOP
Microcephaly, increased nuchal fold, unilateral talipes, cerebellar hypoplasia, molar tooth sign, cardiomegaly, renal pelvis dilatation and dysplastic renal cortex, also had ureteric dilatation, oligohydramnios	PQBP1 (NM_001032381.1): c.155G>A p.(Trp52*) hemi mat	V	Renpenning syndrome	TOP
NIHF	EPHB4 (NM_004444.4): c.2191C>G p.(Leu731Val) het mat	IV	Capillary arteriovenous malformation	CP, LB
NIHF, polyhydramnios, dilated right atrium	FLT4 (NM182925.4): c.3821A>T p.(Asp1274Val) het <i>dn</i>	IV	Milroy disease	CP, LB
bilateral SVC, bicoronal craniosynostosis, FGR, unilateral pelvic kidney, small other kidney, prefrontal edema	KMT2D (NM_003482.3): c.6827del p.(Pro2276Hisfs*10) het <i>dn</i>	V	Kabuki syndrome	TOP
3 rd trimester bilateral pleural effusions 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 <i>dn</i>	V	Noonan syndrome	TOP
Cystic hygroma, VSD, micrognathia, bilateral talipes, absent corpus callosum, hypoplastic thorax	KAT6B (NM-012330.3) : c.5238C>A p.(Cys1756*) het <i>dn</i>	V	SBBYSS syndrome	Opted for TOP, had IUD prior
Bilateral CLP, AVSD	DVL3 (LRG_1269): c.1745del G p.Gly582Alafs*86 het <i>dn</i>	IV	Robinow syndrome	CP, LB
Polyhydramnios, posterior fossa anomaly, bilateral hydronephrosis, brachiocephaly. Maternal historical diagnosis 'Noonan Syndrome'	CNOT1 (NM_016284.4): c.76C>T p.(Arg26*) het <i>mat</i>	V	CNOT1 neurodevelopmental disorder	CP, LB

Table 1. Causative exome sequencing findings based upon prenatal phenotype

[ACMG, American College of Medical Genetics and Genomics and the Association for Clinical Genomic Science, 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, nonimmune hydrops fetalis; NND, neonatal death; pat, paternally inherited; SVC, superior vena cava; TOP, termination of pregnancy; VSD, ventricular-septal defect].

Discussion

In this Liverpool cohort pES provided an antenatal genetic diagnosis in ¼ fetuses. Our findings suggest that after tertiary Fetal Medicine counselling ¼ women with a fetus with either multiple anomalies or non-immune hydrops will have late TOP, whether pES is performed or not. However, when a monogenic cause for fetal anomalies is identified by pES women are more likely to have late TOP than when the result is non-informative.

There are a paucity of papers which report the impact of pES on late termination of pregnancy. And, at present, the proportion of TOPs performed after 24-weeks under Clause E in England and Wales has not changed from 2019 and 2020, since the introduction of R21 (0.13% vs. 0.11%).

In all cases in this cohort significant input was provided from a Clinical Geneticist experienced in prenatal evaluation, with ongoing MDT case discussions. Where pES identified a causative variant, women received joint, individualised Genetic, Fetal Medicine and Neonatal counselling. There is some evidence that formalised multi-disciplinary prenatal genetic clinics, may further optimise diagnostic yield. We welcome the much-anticipated results of the one-year audit of R21 findings, as well as the findings of the Optimising Exome PREnatal Sequencing Services (EXPRESS) study to provide further clarity on the implementation of the R21 pathway.

Conclusion

This study demonstrates the impact of pES on women's decision-making, particularly the likelihood of choosing TOP beyond 22 weeks gestation. As the R21 pathway, inclusion criteria, turnaround time and coverage continue to develop we urge clinicians and policymakers to consider the value of training in early detection of anomalies, robust guidance for late termination of pregnancy and support for couples and families.

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