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Inherited variants in autosomal dominant disease genes are a significant cause of fetal structural anomalies

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ABSTRACT

Background Monogenic disorders are a major cause of fetal structural anomalies. Most genetic diagnoses involve de novo, biallelic or X linked variants; however, inherited variants in autosomal dominant disease genes have been detected across multiple studies. The overall contribution of such variants to fetal structural anomalies is unclear and variant filtering strategies may exclude them. In this study, we aimed to characterise the inherited variants in autosomal dominant disease genes detected by prenatal exome sequencing in a large, well-phenotyped cohort.

Methods The outcomes of prenatal exome sequencing for fetuses with structural anomalies referred to our laboratory from April 2019 to February 2025 were reviewed.

Results Prenatal exome sequencing was carried out in 1185 fetuses, resulting in a diagnosis in 30.0% of cases. Autosomal dominant disorders accounted for 59.9% of diagnoses and a risk of recurrence was identified for 19.2% of these. Autosomal dominant conditions with an increased risk for recurrence were therefore identified in 3.5% of fetuses referred for sequencing, and accounted for 11.5% of prenatal exome sequencing diagnoses. Recurrent diagnoses involving inherited variants included rasopathies and type I/II collagen disorders.

Conclusion Inherited variants in autosomal dominant disease genes are a significant contributor to fetal structural anomalies and may have implications for parents' own health as well as management of the current pregnancy and reproductive options. The requirements for genomic counselling, clinical assessment and genetic testing of parents and family members following an inherited finding must be taken into account when planning delivery of a prenatal sequencing service.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Monogenic disorders are a major cause of fetal structural anomalies.
⇒ The majority of genetic diagnoses involve de novo, biallelic or X linked pathogenic variants; however, inherited heterozygous pathogenic variants in autosomal dominant disease genes have been detected across multiple prenatal sequencing studies.

WHAT THIS STUDY ADDS

⇒ Inherited variants in autosomal dominant disease genes are a significant contributor to fetal structural abnormalities, even for pregnancies where there is no obvious family history.
⇒ Such variants are found in a wide range of genes, including rasopathy genes and type I/II collagens.
⇒ The detection of an inherited variant may necessitate additional clinical assessment and genetic testing in parents and family members.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Parents should be counselled about the possibility of inherited variants and potential implications for their own health when being consented for prenatal sequencing.
⇒ Assessment of parental phenotypes and family history at the point of referral is helpful to identify cases with suspicion for an inherited dominant disorder, and findings from this assessment should be communicated to the testing laboratory.
⇒ Laboratories should review inherited variants relevant to the fetal phenotype, particularly for genes with known reduced penetrance.

INTRODUCTION

A fetal structural anomaly is detected by routine prenatal ultrasound scanning in approximately 3% of pregnancies.[1] The use of prenatal exome sequencing (pES) to investigate the aetiology of fetal structural anomalies was first described around 10 years ago and pES is increasingly being adopted as a diagnostic test during pregnancy.[2] pES offers an increased diagnostic rate over chromosomal microarray and karyotype analysis, dependent on the nature of the anomalies detected.[3] Results from prenatal sequencing aid decision-making and management of pregnancy, birth and the neonatal period, as well as informing genomic counselling for future pregnancies.[4–7]

A trio design, sequencing the fetus and both parents, is often employed in pES in order to leverage parental genotypes for variant filtering.[8] The majority of diagnostic findings in prenatal sequencing are expected to be de novo variants causative of dominant disorders or inherited variants causative of recessive disorders, as is the case

for developmental disorders diagnosed postnatally. However, inherited pathogenic variants in dominant disease genes that were causative of the fetal phenotype were noted in early pES studies in small cohorts.[9 10] Subsequent pES studies in larger cohorts have confirmed that inherited variants in dominant disease genes are a recurrent cause of fetal anomalies.[11–13] However, the overall contribution of such variants to fetal anomalies is unclear, and they may be under-reported in the literature due to exclusion of inherited variants in trio-based variant filtering strategies.

Rapid pES for fetuses with multiple multisystem major structural or selected other abnormalities was introduced into clinical practice in the UK National Health Service in 2020 and is carried out by two laboratories. In this study, we review the results of pES carried out at the West Midlands Genomics Laboratory up to February 2025 to establish the contribution of inherited variants in autosomal dominant disease genes to fetal structural anomalies in this cohort.

METHODS

Patients

A retrospective analysis of pES results from 1185 pregnancies with fetal structural abnormalities referred to the West Midlands Genomics Laboratory between April 2019 and end of February 2025 was carried out. Fetuses had a range of structural abnormalities meeting the inclusion criteria set out by the National Genomic Test Directory.[14] These criteria were updated during the study period. Eligibility for pES is determined by a multidisciplinary team that includes clinical geneticists and fetal medicine experts. Venous blood samples were collected from both parents where available.

Sequencing and data analysis

A negative aneuploidy test (via quantitative fluorescence-PCR) was required prior to requesting pES. Chromosomal microarray was carried out prior to or in parallel with pES by the laboratory local to the referring fetal medicine centre. Trio analysis was carried out on coding and splice regions of a panel of prenatally relevant genes, which was reviewed and updated during the study period.[15] In addition, rare variants in the fetus (minor allele frequency <0.0005) were reviewed for concordance with phenotype. Variants were classified in accordance with guidelines set out by the American College of Medical Genetics and Genomics and the Association for Clinical Genomic Science.[16 17] Variants were not confirmed by Sanger sequencing except in specific cases. Parental relationships were confirmed by analysis of microsatellite markers. For apparently de novo variants, a check for inheritance of multiple rare variants from each parent was made to ensure sample identity. Variants were reported if relevant to the referral reason and classified as pathogenic or likely pathogenic. Variants of uncertain significance relevant to the referral reason, single heterozygous (likely) pathogenic variants in autosomal recessive disease genes, and incidental findings were reported in some cases following multidisciplinary team discussion. At least 99% of coding regions within the virtual gene panel were covered at a depth of at least 20×.

RESULTS

Overview of the cohort

Up to the end of February 2025, pES was carried out in our laboratory for 1185 fetuses (figure 1). Sequencing was initiated during the pregnancy in 87.4% of cases, and following a pregnancy loss or termination in 12.6%. Analysis was carried out as a trio with both parents in 93.2% of cases, and as a duo with one parent or as a singleton in 6.8%. A pathogenic or likely pathogenic finding considered to fully or partially explain the ultrasound scan or postmortem findings was identified in the initial analysis of a virtual panel of genes known to be related to fetal structural anomalies in 27.3% of cases. A diagnosis was made in an additional 1.8% of cases through reclassification of a variant of uncertain significance in light of new evidence. Further analysis of the exome data (either gene-agnostic analysis or analysis of specific additional genes) was carried out on clinician request in selected cases and resulted in diagnoses in a further 0.9% of cases. The overall diagnostic yield from pES was therefore 30.0%, including two cases with dual diagnoses.

Inherited variants in autosomal dominant disease genes

Among 357 diagnoses of monogenic disorders, 59.9% were autosomal dominant (AD) conditions, 33.1% were autosomal recessive and 7.0% were X linked (table 1). Parental samples were available to determine inheritance in 95.8% of AD disorder cases. In 76.6% of cases with a variant in an AD disease gene, the variant appeared to have arisen de novo. In 19.2% of cases with a diagnosis of an AD condition (41 cases across 39 families), an increased risk for recurrence was identified (table 2, online supplemental tables 1 and 2). In 35 cases, the variant was definitively inherited from a parent, including six parents in whom mosaicism was clearly detected. In one further case, the variant was not detectable in either parent, but parental mosaicism is presumed as the variant was also detected in a previous affected fetus of the couple. In addition, there were five cases where the variant was detected in a small number of sequencing reads in one parent, consistent with low-level parental mosaicism, but the possibility of a sequencing artefact could not be excluded. An AD condition with increased risk for recurrence was therefore identified in 3.5% of fetuses referred for pES, and such conditions accounted for 11.5% of all pES diagnoses.

Autosomal dominant disease genes with inherited variants

Inherited variants were detected in 26/85 AD disease genes with diagnostic findings in our cohort. Inherited variants were more frequent within certain groups of AD disease genes (table 3). Genes of the Ras signalling pathway represent the most common group of AD disease genes in our cohort, accounting for around one in five of all AD disorder diagnoses and one in five inherited variants in AD disease genes. Type I/II collagen genes and transcription factor genes, as well as genes associated with (lymph)angiogenesis and holoprosencephaly, appear to be more frequently represented among inherited variants compared with AD diagnoses overall. In contrast, genes encoding chromatin modifiers, including *CHD7*, as well as the fibroblast growth factor receptor genes *FGFR2* and *FGFR3*, were among the most common AD disorder diagnoses overall; however, variants in these genes were never inherited in our cohort. Inherited variants in AD disease genes accounted for 12.9% of diagnoses among 235 fetuses with oedema phenotypes (hydrops, ascites, pleural/pericardial effusion; overall diagnostic yield 36.2%) and 17.3% of diagnoses among 242 fetuses referred with skeletal dysplasia or short long bones (overall diagnostic yield 43.0%).

Previous prenatal sequencing studies have reported inherited diagnostic variants in 18 of the 26 AD disease genes with inherited variants in our cohort (online supplemental table 3 and online supplemental references). Inherited variants in *FBN1* have been reported in the prenatal setting as incidental findings. For the remaining seven genes, there are to our knowledge no previous reports of inherited variants in the prenatal setting;

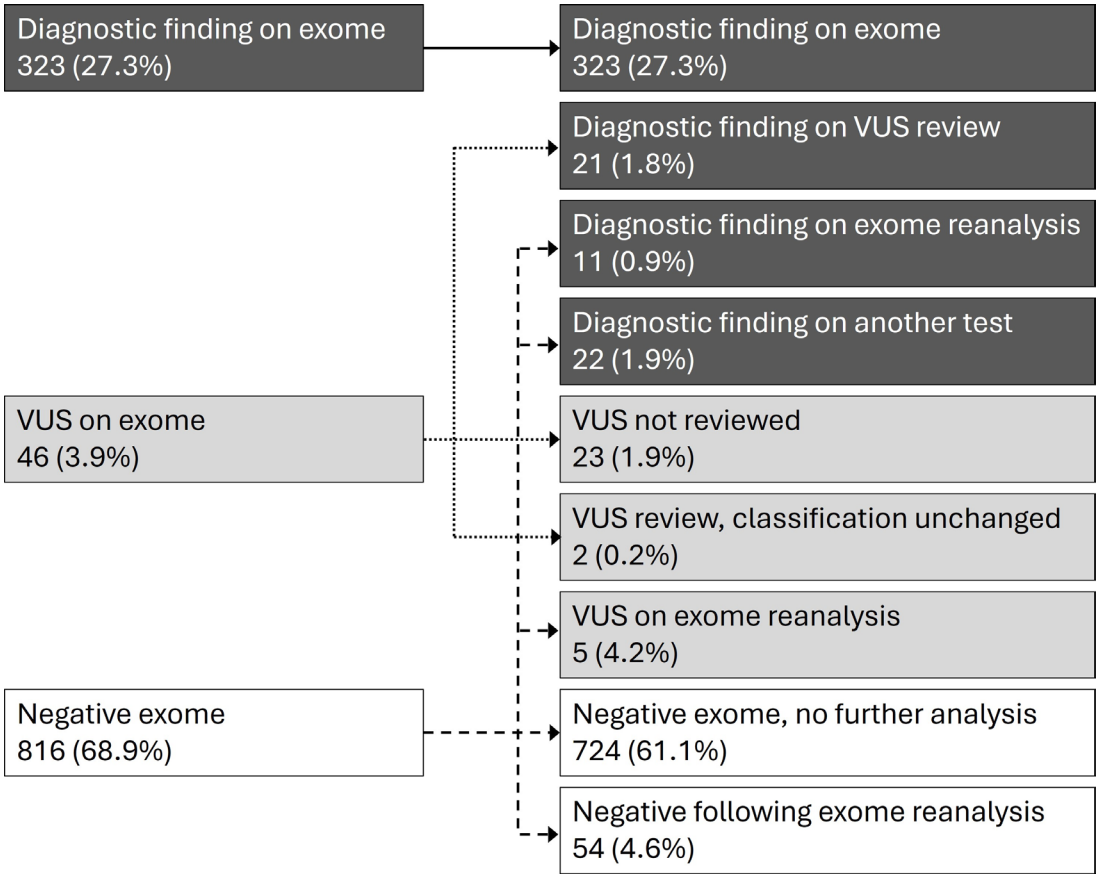

Figure 1 Outcomes of prenatal exome sequencing analysis. Outcomes of prenatal exome sequencing analysis in 1185 fetuses. Diagnostic findings comprise pathogenic or likely pathogenic variants considered to wholly or partially explain the fetal phenotype. Variants of uncertain significance (VUS) were reported if relevant to the phenotype following multidisciplinary team discussion. VUS were reviewed where additional information was received regarding the prenatal or postnatal phenotype, or regarding the variant itself, which might permit reclassification. Negative exome cases include cases with reported incidental findings or reported heterozygous variants in autosomal recessive disease genes where no second variant has been identified. Reanalysis of exome data included gene-agnostic reanalysis or reanalysis for specific genes or gene panels, as requested by the clinician. Other tests resulting in diagnostic findings included microarray, genome sequencing and methylation analysis for imprinting disorders.

however, variants in these genes identified in paediatric patients have been reported to be inherited from an unaffected or mildly affected parent.

Nine of the AD disease genes with inherited variants in our cohort are recorded as also being associated with recessive disease in the OMIM, Gene2Phenotype or ClinGen databases (*ALPL, COL1A2, COL2A1, FBN1, MYH7, PIEZO1, PKD1, POLR1A* and *TCF12*). In all fetuses with variants in these genes, there was no evidence of a second pathogenic sequence variant or CNV, and the variant type and family history were consistent with AD disease, although we cannot fully exclude the possibility of an undetected second variant.

Fetal phenotypes and family history

An inherited variant in an AD disease gene is likely to fully explain the fetal phenotype in 32 families, while in five cases the variant is likely to partially explain the phenotype, and in two cases the relevance of the variant to the phenotype is uncertain (table 2, online supplemental table 1). In around half of families (21/39), a familial monogenic disorder was considered possible in the fetus at the time of referral for pES, based on parental phenotypic assessment and/or family history (online supplemental table 1). In two families, a genetic diagnosis of an AD condition was already established in one parent. In 12 families, clinical features relevant to the fetal phenotype were observed in a parent (and also in further family members in four families), but with no previous genetic diagnosis. In the case of one fetus with holoprosencephaly and unaffected parents, a maternal aunt was affected. For four further families, a similar fetal phenotype had been observed in a previous pregnancy.

Following detection of an inherited variant in an AD disease gene, retrospective review of referral forms showed that relevant clinical features were documented in 15 transmitting parents (including four with mosaicism), while clinical review of parents in light of the pES result identified relevant phenotypes in a further eight (including one with mosaicism) (online supplemental table 1). Parental phenotypes identified on clinical review were typically subtle and non-specific (such as mild short stature or birthmarks), or were detectable only through laboratory investigations and had therefore not been documented at the time of referral. Relevant clinical features have therefore been identified in 22 transmitting parents (59%), whereas 16 parents (41%) are not known to have any relevant phenotype. Seven of the apparently unaffected parents have mosaic variants, which may account for the lack of an obvious phenotype. It is possible that mosaicism is also present in additional parents, despite the variants appearing to be heterozygous in blood, due to variation in mosaicism levels between tissues. Incomplete penetrance is likely to explain the absence of a phenotype in at least some parents. For example, in families with *EPHB4*

Table 1 Inheritance pattern for diagnostic variants from prenatal exome sequencing

Inheritance pattern	Diagnoses
Autosomal dominant	214 (59.9%)
Presumed de novo*	164
Inherited from parent with no evidence of mosaicism	29
Inherited from a parent with detectable mosaicism†	6
Possible low-level mosaicism in parent‡	5
Presumed germline mosaicism§	1
Unknown inheritance	9
Autosomal recessive	118 (33.1%)
Homozygous, biparental inheritance confirmed	66
Homozygous, uniparental disomy confirmed	2
Homozygous, only one parent tested and is a carrier	3
Compound heterozygous, biparental inheritance confirmed	46
Compound heterozygous, only one parent tested and is a carrier	1
X linked	25 (7.0%)
Hemizygous in male fetus, maternally inherited	7
Hemizygous in male fetus, de novo	3
Heterozygous in female fetus, de novo	15
Total	357

*Based on trio pES in 163 cases, and duo pES with subsequent Sanger sequencing of the second parent in one case. Germline mosaicism is not excluded.
†Mosaicism detected in parental blood ES data with variant allele fraction of 5%–25%. Parental mosaicism was confirmed by Sanger sequencing in selected cases.
‡Variant detected in a small number of reads in parental blood ES data (variant allele fraction <2%), but not confirmed by an orthogonal method.
§Variant not detected in parental blood samples but recurrent in two pregnancies.
pES, prenatal exome sequencing.

and *SHH* variants, a relevant phenotype was noted in another family member even though the transmitting parent was apparently unaffected, consistent with previous reports of incomplete penetrance for these disorders.[18 19 20] It is possible that some apparently unaffected parents do have mild features, as some may not have undergone detailed clinical review, or findings may not have been communicated to us.

Impact of inherited findings

As our laboratory processes pES referrals from multiple UK regions and Ireland, we do not have complete information regarding outcomes and family follow-up subsequent to detection of an inherited variant in an AD disease gene. Pregnancy outcomes could be ascertained in 21 families and comprised 14 live births (67%) and seven terminations of pregnancy (33%). Pregnancy outcome data are not routinely sought following pES, but were available for 183 further pregnancies, with outcomes of 60% live birth, 33% termination of pregnancy and 8% pregnancy loss. Among 22 cases with known pregnancy outcome and a de novo variant in an AD disease gene, outcomes were 32% live birth, 55% termination of pregnancy and 14% pregnancy loss. Therefore in this small sample, inherited variants in AD disease genes appear to be associated with lower rates of termination of pregnancy and of pregnancy loss compared with de novo variants.

Some neonates were noted to have clinical features that were explained by the pES result and had not been detectable on prenatal ultrasound scan, such as facial features of Noonan syndrome associated with a *PTPN11* variant, and capillary malformations associated with *RASA1* variants. Results of pES informed postnatal management, for example, referral to endocrinology for a neonate with a *PLAG1* variant, and referral for neuroimaging for infants with *RASA1* variants.

In several families, pES provided a genetic diagnosis for a lifelong condition in the transmitting parent, including variants in *CNOT1, PLAG1*[21] and *PTPN11* (online supplemental table 1). In some cases, the pES result prompted referral of the transmitting parent for specialist assessment or imaging relevant to managing their own health, including referral to an inherited cardiovascular conditions clinic (*MYH7*), to endocrinology (*PLAG1*), for cardiac scan (*PTPN11*), for imaging to monitor for intracranial arteriovenous malformations (*RASA1*) and for neuroimaging for developmental brain abnormalities (*TUBB2B*). Parents and/or other relatives were referred for specialist assessment or imaging as a result of the pES result in 6/11 families seen by our local clinical genetics service. Cascade testing has been carried out in 3/11 families seen by our local clinical genetics service, which has identified other affected family members in two families.

The chance of recurrence of an AD disorder in future pregnancies is up to 50% if the variant is detected in a parent. Invasive prenatal diagnosis in subsequent pregnancies has been carried out at our laboratory for families with variants in *COL1A1, COL1A2* and *PTPN11*, with the fetuses being unaffected in all cases. Prenatal diagnosis for a *ZIC2* variant was declined following normal ultrasound scan. Development of a bespoke non-invasive prenatal diagnosis (NIPD) assay is an option for the 16 families in our cohort with no evidence of the variant in maternal blood, but cannot be offered for 23 families with maternally transmitted variants. We have validated bespoke NIPD assays for two families with variants in *COL1A1* and *KRAS*. Development of a bespoke NIPD assay for a *ZIC2* variant was unsuccessful due to homology between the target region and other genomic regions. Preimplantation genetic testing (PGT-M) is currently authorised by the UK Human Fertilisation and Embryology Authority for conditions associated with 21/26 genes with inherited variants in our cohort (conditions associated with *CNOT1, PLAG1, POLR1A, TUBB2B* and *ZFHX4* have not yet been considered for authorisation). We are aware of two families in our cohort who have pursued PGT-M for inherited variants in *COL2A1* and *EPHB4* detected by pES.

DISCUSSION

The landscape of inherited variants

Our findings demonstrate that inherited variants in AD disease genes are a significant cause of fetal structural anomalies, potentially accounting for over 10% of diagnostic findings in our large, well-phenotyped cohort. The transmitting parent may be clearly affected themselves (with or without a genetic diagnosis), may have mild or unrecognised clinical features different to those in the fetus due to variable expressivity or may be unaffected due to incomplete penetrance or mosaicism. Although not observed in our cohort, a transmitting parent might also be unaffected due to parent-of-origin effects, as has been reported for maternally transmitted *CDKN1C* variants and paternally transmitted *MAGEL2* variants detected by prenatal sequencing.[13 22–25] Among 27 families in our cohort in which the variant appeared to be constitutional in the transmitting parent, the variant was transmitted by the mother in 18 families and by the father in nine families, suggesting that maternal transmission might be more common. However, in view of the limited sample size, further studies are needed to confirm if this is the case and the reasons for any such effect. Among transmitting parents with constitutional variants in our cohort, the proportion of individuals with clinical features is similar between males and females.

Table 2 Autosomal dominant disorder diagnoses associated with inherited variants

Family	Phenotype	Gene	Disorder	Parental mosaicism	Additional information
1	Skeletal	*ALPL* (MIM: 171 760)	Hypophosphatasia	No	Affected twins with same variant
2	Brain	*CNOT1* (MIM: 604 917)	Vissers-Bodmer syndrome	No	
3	Skeletal	*COL1A1* (MIM: 120 150)	Osteogenesis imperfecta	No	
4	Skeletal	*COL1A1* (MIM: 120 150)	Osteogenesis imperfecta	Yes	
5	Skeletal	*COL1A1* (MIM: 120 150)	Osteogenesis imperfecta	Possible low level	
6	Skeletal	*COL1A2* (MIM: 120 160)	Osteogenesis imperfecta	Yes	
7	Skeletal	*COL1A2* (MIM: 120 160)	Osteogenesis imperfecta	No	
8	Skeletal	*COL2A1* (MIM: 120 140)	Type II collagen disorders	No	
9	Skeletal	*COL2A1* (MIM: 120 140)	Type II collagen disorders	Possible low level	
10	Skeletal	*COL2A1* (MIM: 120 140)	Type II collagen disorders	No	
11	Skeletal	*COL2A1* (MIM: 120 140)	Type II collagen disorders	Yes	
12	Oedema	*EPHB4* (MIM: 600 011)	Capillary malformation-arteriovenous malformation 2	No	
13	Multisystem	*FBN1* (MIM: 134 797)	Marfan syndrome	No	Partially explains phenotype. Known familial variant
14	Oedema	*KRAS* (MIM: 190 070)	Noonan syndrome 3	Possible low level	
15	Multisystem	*KRAS* (MIM: 190 070)	Noonan syndrome 3	Possible low level	
16	Cardiac	*MYH7* (MIM: 160 760)	Cardiomyopathy and skeletal myopathy	No	
17	Cardiac	*MYH7* (MIM: 160 760)	Cardiomyopathy and skeletal myopathy	No	
18	Multisystem	*PIEZO1* (MIM: 611 184)	Dehydrated hereditary stomatocytosis with or without pseudohyperkalaemia and/or perinatal oedema	No	Partially explains phenotype. Additional de novo likely pathogenic *GLI2* variant
19	Multisystem	*PKD1* (MIM: 601 313)	Polycystic kidney disease 1	No	Partially explains phenotype. Known familial variant
20	Skeletal	*PLAG1* (MIM: 603 026)	Silver-Russell syndrome 4	No	
21	Multisystem	*POLR1A* (MIM: 616 404)	Acrofacial dysostosis, Cincinnati type	No	Partially explains phenotype
22	Multisystem	*PTPN11* (MIM: 176 876)	Noonan syndrome 1	No	
23	Multisystem	*PTPN11* (MIM: 176 876)	Noonan syndrome 1	Possible low level	
24	Multisystem	*PTPN11* (MIM: 176 876)	Noonan syndrome 1	No	
25	Multisystem	*RAF1* (MIM: 164 760)	Noonan syndrome 5	No	
26	Oedema	*RASA1* (MIM: 139 150)	Capillary malformation-arteriovenous malformation 1	No	
27	Multisystem	*RASA1* (MIM: 139 150)	Capillary malformation-arteriovenous malformation 1	No	
28	Oedema	*RASA1* (MIM: 139 150)	Capillary malformation-arteriovenous malformation 1	No	Two affected pregnancies with variant
29	Oedema	*RIT1* (MIM: 609 591)	Noonan syndrome 8	No	
30	Skeletal	*RUNX2* (MIM: 600 211)	Cleidocranial dysplasia	Yes	
31	Brain	*SHH* (MIM: 600 725)	Holoprosencephaly 3	No	
32	Oedema	*SOS1* (MIM: 182 530)	Noonan syndrome 4	No	
33	Skeletal	*SOX9* (MIM: 608 160)	Campomelic dysplasia	Presumed germline mosaicism	Variant also detected in a previous affected fetus
34	Multisystem	*TCF12* (MIM: 600 480)	Craniosynostosis 3	No	Uncertain relevance to phenotype
35	Clefting	*TFAP2A* (MIM: 107 580)	Branchiooculofacial syndrome	No	
36	Multisystem	*TUBB2B* (MIM: 612 850)	Cortical dysplasia, complex, with other brain malformations 7	No	Partially explains phenotype. Additional homozygous VUS in *UQCRC2*
37	Multisystem	*ZFHX4* (MIM: 606 940)	Neurodevelopmental disorder	No	Detected on gene-agnostic reanalysis. Uncertain relevance to phenotype
38	Brain	*ZIC2* (MIM: 603 073)	Holoprosencephaly 5	Yes	
39	Brain	*ZIC2* (MIM: 603 073)	Holoprosencephaly 5	Yes	

VUS, variants of uncertain significance.

In our cohort, inherited variants were detected in a wide range of AD disease genes, including some of the most common diagnoses in the cohort overall, particularly rasopathies and collagen I/II disorders, in which variable expressivity is well documented.[26–28] For all of the genes in which we detected inherited variants, there are previous reports of disease-causing variants being inherited from a mildly affected or unaffected parent, providing evidence for variable expressivity and/or incomplete penetrance for all of these genes. Some AD disease genes which recurrently harbour inherited variants in published prenatal sequencing studies are rare or absent in our cohort, predominantly due to the inclusion criteria for pES within the NHS. For example, recurrently affected genes in the literature include those typically associated with isolated cardiac

Table 3 Autosomal dominant disease gene groups with recurrent diagnoses in our cohort

	Cases	Inheritance			% of diagnoses		
Gene or gene group*	Total	De novo	Inherited	Not known	All (total: 357)	All AD (total: 214)	Inherited AD (total: 41)
Ras pathway	49	38 77.6%	8 16.3%	3 6.1%	13.7%	22.9%	19.5%
Collagen I/II	33	23 69.7%	9 27.3%	13.0%	9.2%	15.4%	22.0%
Chromatin modifiers (excluding *CHD7*)	20	18 90.0%	0 0%	2 10.0%	5.6%	9.3%	0%
Transcription factors	16	10 62.5%	6 37.5%	0 0%	4.5%	7.5%	14.6%
CHD7	15	14 93.3%	0 0%	16.7%	4.2%	7.0%	0%
FGFR2/3	15	14 93.3%	0 0%	16.7%	4.2%	7.0%	0%
Actin/Myosin and interactors	12	10 83.3%	2 16.7%	0 0%	3.4%	5.6%	4.9%
Tubulins	9	8 88.9%	111.1%	0 0%	2.5%	4.2%	2.4%
(Lymph)angiogenesis	7	2 28.6%	5 71.4%	0 0%	2.0%	3.3%	12.2%
RNA biology	6	4 66.6%	2 33.3%	0 0%	1.7%	2.8%	4.9%
PI3K/AKT/mTOR	5	5 100%	0 0%	0 0%	1.4%	2.3%	0%
Holoprosencephaly	3	0 0%	3 100%	0 0%	0.8%	1.4%	7.3%
Others	24	18 75.0%	5 20.8%	14.2%	6.7%	11.2%	12.2%

*Ras pathway: *PTPN11, RAF1, BRAF, RIT1, HRAS, KRAS, NRAS, CBL, SHOC2, LZTR1, SOS1*. Collagen: *COL1A1, COL1A2, COL2A1*. (Lymph)angiogenesis: *RASA1, EPHB4, FLT4*. Holoprosencephaly: *SHH, ZIC2*. Tubulins: *TUBA1A, TUBB, TUBB2B*. Chromatin modifiers: *ANKRD11, ARID1A, ARID1B, CHD7, CREBBP, KANSL1, KAT6B, KMT2A, KMT2D, NSD1, SETBP1, SMARCA4*. Actin/Myosin: *ACTA1, ACTB, ACTG2, FLNB, MYH3, MYH7, MYH10*. Transcription factors: *FOXF1, FOXG1, GATA4, GLI2, PLAG1, RUNX2, SOX9, TCF12, TFAP2A, TP63, WT1, ZFHX4*. RNA biology: *CNOT1, EFTUD2, POLR1A, SF3B4, SNRPB, SON*. PI3K/AKT/mTOR pathway: *CCND2, mTOR, PIK3CA*. Others: *ALPL, CLTC, COL4A1, DLL1, DVL3, DYNC1H1, DYRK1A, FBN1, KIF11, NIPBL, PIEZO1, PIEZO2, PKD1, PKD2, PPP2R1A, PTCH1, RHOA, SLC25A24, TRPV4, TSC2*.
AD, autosomal dominant; CHD7, chromodomain helicase DNA-binding protein 7; FGFR2/3, fibroblast growth factor receptor 2/3; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase.

defects (eg, *ELN*, *NOTCH1*, *GATA4*), isolated kidney defects (eg, *HNF1B*, *GREB1L*, *PKD1*, *PKD2*) or highly specific clinical presentations amenable to targeted testing (eg, *TSC1*, *TSC2*), which may not meet the current eligibility criteria for pES within the NHS.[12 13 29–43] If eligibility criteria were extended to abnormalities affecting a single system, then inherited variants in AD disease genes might increase as a proportion of total diagnoses.

Variant filtering, prioritisation and interpretation

Many pES variant filtering pipelines employ trio-based inheritance filtering to reduce the number of variants requiring interpretation, particularly where gene-agnostic analysis is carried out. However, this study and others support inclusion of a partially penetrant filter setting for at least a subset of genes. In view of the increasing number of genes recognised to be associated with fetal anomalies, and the use of gene-agnostic analysis, strategies for prioritisation of rare inherited variants are required. These may include whitelists of known pathogenic variants, predictive scores for impact on splicing and protein function, tools such as Exomiser which incorporate phenotype information and artificial intelligence-based algorithms.

The interpretation of inherited variants in AD disease genes may be challenging, particularly for novel variants without a clear loss-of-function effect, and where the gene-disease association is characterised by incomplete penetrance and/or variable expressivity. There may be insufficient evidence available to reach a pathogenic classification at the time of initial analysis, particularly in the prenatal setting, where phenotypic information is limited. Multidisciplinary input to obtain detailed fetal and parental phenotype data was essential for variant classification in some of our cases. Where accurate classification is likely to require evidence from postnatal phenotyping, extended family studies or experimental data on RNA splicing or protein function, it may not be possible to reach a final classification during pregnancy. In three families in our cohort, an inherited variant in an AD disease gene was originally classified as a VUS, and only later reclassified as likely pathogenic due to additional evidence (online supplemental table 2). The decision whether to report an inherited VUS in pregnancy requires discussion with the wider clinical team.

Parental mosaicism

With the use of trio next-generation sequencing, parental mosaicism is more readily detectable compared with traditional methods. Some variants which were mosaic in a parent in our cohort were annotated as de novo by the bioinformatics pipeline, highlighting the importance of manually reviewing parental read data to identify cases with an increased risk of recurrence. In five parents, we were not able to ascertain from the exome data whether the small number of variant reads represented low-level mosaicism or a technical artefact. However, the presence of clinical features of osteogenesis imperfecta (OI) in one parent with potential mosaicism for a *COL1A1* variant, together with suspected OI in their previous child (online supplemental

table 1), suggests that detection of a variant in even a very small number of parental reads may be indicative of genuine mosaicism and warrants reporting to facilitate further clinical assessment of the parent, and potentially genetic testing of additional samples. A buccal sample was received from one of the five parents with potential low-level mosaicism, but the variant could not be confirmed in this sample.

Furthermore, the absence of a variant from parental read data does not completely exclude the possibility of parental mosaicism. A *SOX9* variant detected by pES in our cohort was undetectable in parental blood but was identified in a previous affected fetus of the couple by Sanger sequencing, providing evidence for parental mosaicism and a recurrence risk of up to 50%. Based on estimates of parental mosaicism rates for apparently de novo variants of 0.3%–9%,[44 45] there may be additional families in our cohort with apparently de novo variants who are at high risk of recurrence. To account for the possibility of parental mosaicism for apparently de novo variants, genomic counsellors may give a generalised recurrence risk of 1%–2% (or higher for genes where a relatively frequent occurrence of parental mosaicism is established, such as type I/II collagens).[46 47] However, the true recurrence risk for any couple is either significantly higher, if mosaicism is present, or negligible, if it is not. Within routine care, there are few options available to investigate parental mosaicism to aid reproductive decision-making. If mosaicism is suspected, additional parental tissues may be sequenced, but non-invasive sampling is generally limited to buccal epithelial cells. Parental mosaicism may be detected in the course of validation of a bespoke NIPD assay[48]; however, this is still limited to analysis of blood. The potential paternal mosaicism for a *KRAS* variant in one family in our cohort could not be confirmed or excluded by NIPD assay validation. In addition, bespoke NIPD assay validation cannot be offered if the variant of interest is present in the maternal blood at any level. Due to the lack of further testing options, the true recurrence risk remains unresolved for most couples where the variant is not detected in parental blood, or is present in only a small number of sequencing reads. Recently, an approach to achieving a personalised recurrence risk assessment has been described, which combines deep sequencing of multiple tissues with haplotyping to determine the parental origin of the variant, with sequencing of sperm used to quantify recurrence risk for paternal variants.[45 48] Wider availability of such testing would enable more accurate genomic counselling as well as targeting of prenatal diagnosis to couples at higher risk, avoiding unnecessary invasive testing or validation of bespoke NIPD assays for low-risk couples.[49 50]

The patient pathway

Currently within the NHS, eligibility for pES is considered by a clinical geneticist in conjunction with fetal medicine specialists. Our findings show that clinical genetics input has been valuable in identifying parental phenotypes and family history that are suggestive of a familial condition, including cases where the fetal and parental phenotypes are different, but within the phenotypic spectrum of the same condition. Any move towards mainstreaming of referrals, with reduced clinical genetics involvement, should ensure that information on parental phenotypes and family history continues to be collected and communicated to the laboratory to aid in the interpretation of pES data.

Pretest genomic counselling for all parents considering prenatal sequencing must include the possibility of an inherited variant, which may have implications for the parent's own health as well as reproductive options. Among our families, there were a small number of parents who had been suspected to have a genetic condition from childhood but only received a genetic diagnosis as a result of prenatal sequencing. Ideally, suspected genetic disorders in parents should be investigated prior to pregnancy to enable the development of a targeted test for any familial variant. However, where the parent's features only come to light during investigation of fetal anomalies, rapid trio pES may increase the range of choices available for management of pregnancy and birth in comparison with pursuing sequential investigations in the parent and fetus. In addition, it may be difficult to assess if prenatal scan findings reflect the familial condition or an independent diagnosis, making comprehensive testing preferable. Given the higher prior probability of a monogenic cause for fetal anomalies if a parent or other close relative is suspected to have a monogenic disorder, pES might be considered appropriate even where the fetal phenotype does not strictly meet eligibility criteria (as is already the case in the NHS for consanguineous couples, and couples with recurrence of a fetal phenotype in multiple pregnancies).

Two parents in our cohort had a genetic diagnosis of an AD condition at the time of referral for pES. Known familial variants raise additional issues in prenatal sequencing. Parents may not wish for specific testing for the familial condition in the fetus if this would not affect their decision-making around the pregnancy; however, the familial variant is likely to be detected through routine analysis if the gene is included in the gene panel, or if a gene-agnostic approach is taken. As the prenatal presentations of even well-known conditions are not necessarily well described, it may be unclear if the familial variant is contributing to the fetal phenotype. In the case of the familial *FBN1* and *PKD1* variants in our cohort, it is likely that these explained some, but not all of the findings on ultrasound scan.

Following detection of an inherited variant in an AD disease gene by pES, genomic counselling is essential to explain the implications of the finding for reproductive choices and the parent's own health. The psychological impact of such findings has not been explored. Further research is required to determine how pregnancy outcomes differ for pregnancies with inherited compared with de novo variants in AD disease genes. An inherited finding might be reassuring to parents and support a decision to continue the pregnancy, but the genetic result must be considered in the context of scan findings and other investigations. Within our cohort, families used the information about an inherited variant in an AD disease gene to pursue both invasive and NIPD, as well as PGT-M. The information from pES was also used for further investigation of phenotypes in transmitting parents, including referrals for specialist assessment or imaging to identify any associated phenotypes requiring further management.

CONCLUSION

The high frequency of inherited variants in AD disease genes in fetuses with structural anomalies has wide-ranging implications for the delivery of a prenatal sequencing service, necessitating pre-test and post-test genomic counselling, assessment of parental phenotypes and family history at the point of referral, appropriate analysis and interpretation of sequencing data and further clinical assessment and genetic testing of parents and other family members following prenatal sequencing results.

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