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ARTICLE

Prenatal Variants of Uncertain Significance (VUS): to report or not to report?

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Prenatal Exome Sequencing (pES) has a significant diagnostic yield but time pressure and limited phenotypic information make interpretation of Variants of Uncertain Significance (VUS) more challenging than in a postnatal setting. We share our experiences of prenatal reporting of highly suspicious VUS. We retrospectively analyzed pregnancies in which VUS identified by pES were reported to parents during pregnancy in two Dutch academic medical hospitals. During the study period, 31 VUS in 28 genes were reported in 27 pregnancies. Cases were assigned to one of five groups based on consistency of prenatal phenotypes with gene-associated diagnoses. The implications of VUS included clinical evaluation of parental carriers ($N = 4$), additional screening of proband ($N = 2$), influencing parental decision-making ($N = 11$) and/or prompting confirmatory testing ($N = 10$). Reanalysis with currently available data resulted in reclassification of seven variants, five of which were upgraded to (likely) pathogenic. Although we do not recommend routine disclosure, our data suggest that prenatal reporting of VUS can be valuable in exceptional cases. Stringent selection was applied and only a minority of reported VUS was reclassified as (likely) pathogenic. Therefore, a careful individual assessment of each VUS case remains imperative and multidisciplinary meetings should be an integral part of prenatal VUS management.

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INTRODUCTION

An extensive genetic diagnostic workup should be offered when a fetal anomaly is detected, since a genetic diagnosis can significantly impact prognosis. Several years ago, prenatal genetic testing mainly consisted of techniques to detect chromosomal aberrations. Today, the application of Next Generation Sequencing-based (NGS) detection of single nucleotide variants (SNV) in routine prenatal care offers a high diagnostic yield (8–14%) when chromosome and/or Copy Number Variant (CNV) analysis are normal [1–3]. In a previous study [4] involving a majority of cases in which early diagnosis was vital for pre- or perinatal interventions and parental decision making, we demonstrated the significant clinical impact of prenatal Exome Sequencing (pES). However, the higher resolution of NGS inevitably leads to detection of Variants of Uncertain Significance (VUS), which laboratory specialists, clinicians and patients find particularly challenging to interpret, especially in view of short turnaround times (TAT) and incomplete information regarding fetal phenotype. Detecting VUS is now a commonly encountered challenge, with a systematic review estimating rates as high as 8% of identifying VUS with pES [5]. Current international guidelines [6–9] offer little assistance when dealing with prenatal VUS, only stating that some laboratories may choose to report VUS and that this possibility should be discussed with patients during pre- and post-test counseling.

The common goal of diagnostic tools is to provide parents with a clear prognosis or to potentially offer some reassurance when genetic testing is negative. The detection of a variant with an uncertain pathogenicity may inadvertently increase parents' anxiety. Although studies suggest that parents prefer to receive as much information as possible concerning their unborn child [10], reporting uncertain results in a prenatal setting still divides healthcare professionals regarding whether the beneficence of increased autonomy outweighs the maleficence of increased uncertainty [11]. In this report, we describe our experiences of reporting highly suspicious ('hot') VUS in a prenatal setting and validate a previously published flowchart [12] as a useful tool for navigating challenging variants.

MATERIALS AND METHODS

In the Netherlands, offering pES is now part of the routine diagnostic workup in pregnancies with congenital anomalies suspected of an underlying Mendelian disorder. If no Non-Invasive Prenatal Test (NIPT) was performed, aneuploidies were first excluded before initiation of pES. CNV analysis was done in parallel with pES. Details on laboratory procedures can be found in the Supplementary materials. During pretest counseling, the possibility of an uncertain result was discussed with patients. Class 3 variants, classified according to the American College of Medical Genetics and Genomics [13] (ACMG) criteria, are generally not reported in the prenatal setting by the two participating genomic laboratories. However, some VUS seem highly suspicious due to matching phenotype, strong in silico predictions of pathogenic effect in combination with de novo appearance and/or very low frequency in population

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databases, but with insufficient arguments to reach class 4 (likely pathogenic). In case of a highly suspicious class 3 variant, reporting is discussed in a multidisciplinary meeting that includes at least clinical geneticists and laboratory specialists. Other clinical specialists or ethicists may be involved if deemed necessary. When consensus is reached, a 'hot' VUS is reported in exceptional cases.

We retrospectively analyzed all consecutive pregnancies in which a 'hot' VUS identified by pES was reported to parents during pregnancy in two hospitals:

1. Leiden University Medical Centre (LUMC): all prenatally-reported VUS from first implementation of pES in 2017 to 2023 were included (N = 24 cases, out of N = 552 pES cases in total).
2. Erasmus Medical Centre (EMC): to avoid overlap with previous publications [12] only prenatally-reported VUS from 2022 and 2023 were included (N = 3 cases, i.e. cases 11, 18 and 27, out of N = 495 pES cases in total).

Cases in which structural variants were detected in addition to VUS were excluded from this report. A waiver of approval was obtained from the Medical Ethics Committee of both centers (LUMC nWMO-D4-2023-004, EMC 2024-0435).

RESULTS

In total, 31 variants in 28 genes were reported in 27 cases during the study period. All case details are provided in Supplementary table 1, while phenotype and genotype data are summarized in Table 1, with inheritance categories visualized in Fig. 1 and a color-coded overview of all cases in Fig. 2 (based on Shi et al. [14]). For discussion of the variants, cases were grouped based on a (potential) link between the gene-associated diagnosis and the ultrasound anomalies.

Well-established consistency of gene-associated diagnoses with fetal phenotypes

In 10 cases, VUS were detected in genes that matched the fetal phenotypes. Cases 1 and 2 involved compound heterozygous variants in the genes *DNAH11* (OMIM 603339) and *GDF1* (OMIM 602880), respectively. Both genes are associated with ciliopathies and both pregnancies were characterized by congenital heart defects (CHD). In case 1, the parents continued the pregnancy because they felt reassured by a result that was not associated with neurodevelopmental delay (NDD). The parents in case 2 chose termination of pregnancy (TOP) mainly based on the severity of ultrasound anomalies. In cases 3 and 4, pES detected biparental homozygous variants. In case 3, the *NUP155* (OMIM 606694) VUS possibly explained fetal cardiac arrhythmia, and an association of *NUP155* with cardiomyopathy is an indication for continued postnatal cardiac screening. At follow-up at age 7 months, the proband required medication for arrhythmia, thus increasing the likelihood of this variant being pathogenic. In case 4, the identification of a VUS in *SLC12A6* (OMIM 604878) would only partly explain the phenotype if pathogenic. In addition, a single individual in GnomAD was present with this homozygous variant and aged over 50 years, making pathogenicity of this variant less likely. In this case the parents chose TOP based mainly on ultrasound anomalies, with the VUS playing no role in the decision. A hemizygous *FAM50A* variant (OMIM 300453) was detected in case 5. Pathogenic variants in *FAM50A* lead to an X-linked recessive intellectual development disorder with possible cleft lip. In cases 6-9, heterozygous variants in genes associated with autosomal dominant inheritance were identified, all inherited from asymptomatic parental carriers. Cases 6 and 7 involved the genes *IFIH1* (OMIM 606951) and *LZTR1* (OMIM 600574), which are primarily associated with NDD in addition to congenital anomalies. Since functional validation of variants in cases 5 and 6 performed during pregnancy showed no effect on protein, and extended segregation analysis identified the hemizygous *FAM50A* variant in the asymptomatic maternal grandfather, the chances of pathogenicity decreased significantly. Parents felt reassured because of this information and both pregnancies were continued. The pregnancy with the *LZTR1* variant was continued without additional options to further classify the variant. At age 16 months this proband displayed normal development and no characteristics of Noonan syndrome (OMIM 616564). Cases 8 and 9 were affected by CHD for which VUS in *TAB2* (OMIM 605101) and *CRELD1* (OMIM 607170), respectively, may be explanatory. Cardiac imaging of parental carriers was advised, which was normal in both cases. Pregnancies were continued and both probands show normal cognitive and motoric development, although in case 9 the proband has a mild language development delay which is attributed to frequent ear infections. A de novo variant in *EP300* (OMIM 602700) was identified in case 10, a fetus showing CHD. This gene has been linked to several conditions including Rubinstein-Taybi syndrome (MIM#613684) and this additional uncertainty led the parents to opt for TOP. Postnatal dysmorphological examination did not give sufficient confirmation to reclassify the variant.

Possible phenotypic consistency of gene-associated diagnoses with fetal phenotypes

In five cases, variants were detected in genes with associated diagnoses that bore similarities to the ultrasound anomalies but were not identical. In two cases (11, 12), variants in *MYH6* (OMIM 160710) were detected in pregnancies affected by hypoplastic left heart syndrome (HLHS). As *MYH6* is associated with cardiomyopathy and possible atrial septal defects, HLHS could be a yet undescribed phenotypical expansion or might have contributed to the phenotype. In either case, this was an indication for cardiac ultrasound screening of parental carriers. In case 11, the father suffered from coarctation aortae and bicuspid aortic valve, with several other family members harboring the variant and showing a variety of cardiac abnormalities. The proband passed away 7 weeks after birth due to complicated surgical correction. In case 12 the parents did not undergo cardiac ultrasound but they nevertheless felt reassured that this result supported the notion of an isolated anomaly, and at age 2.5 years the proband shows normal development with mild microcephaly (–2.5 SD). In case 13 a paternally inherited variant in *ROBO2* (OMIM 602431) was identified, which is associated with vesicoureteral reflux. The fetus showed multicystic kidney dysplasia and the father had unilateral kidney agenesis. The paternal grandparents underwent a kidney ultrasound that showed no abnormalities but they were not tested for the *ROBO2* variant. The parents were reassured by the paternal phenotype and thus continued the pregnancy.

In two more cases a de novo variant was detected. In case 14, ultrasound showed a ventricular septal defect and pES identified a variant in *CUL3* (OMIM 603136), a gene predominantly associated with NDD and possibly with congenital heart disease. This additional uncertainty led the parents to opt for TOP. A de novo VUS in *PTCH1* (OMIM 601309), which is associated with Gorlin syndrome (OMIM 109400), was detected in case 15. This fetus had megalencephaly, while macrocephaly is part of the known phenotypic spectrum of Gorlin. Other congenital anomalies associated with Gorlin syndrome were ruled out during an ultrasound. At age 4 years this proband shows gross motoric developmental delay with autistic features and macrocephaly, with follow-up management based on a suspicion of Gorlin syndrome.

Case reports describing possible consistency of gene-associated diagnoses with fetal phenotypes

In four cases, VUS were reported in genes for which a matching OMIM phenotype was not yet well established, but literature at the time of reporting suggested (either by single case reports or by preclinical models) that the gene might be responsible for the fetal phenotype. Case 16 concerned compound heterozygous

Table 1. Details on phenotype and genotype including variant reclassification and flowchart validation.

Case nr	GA at pES result	Phenotype	Variant	Associated OMIM diagnosis	ACMG classification			Report according to flowchart [12] including criteria
					At time of reporting	As applied with currently available data	Details on reclassification	
Well established phenotypic consistency of gene-associated diagnoses with fetal phenotypes								
1	21 + 6	Atrioventricular canal defect HP:0006695, Left Isomerism HP:0031854, Abdominal situs inversus HP:0003363	NM_001277115.1(DNAH11):c.384del p.(Phe128Leufs*12), het pat NM_001277115.1(DNAH11):c.10472 G > A p.(Arg3491His), het mat	Primary ciliary dyskinesia type 7 with or without situs inversus	LP VUS (PM2 PM3 PP3)	NA VUS (PM3)		NA Yes, matching the phenotype
2	19 + 0	Transposition of the great arteries HP:0001669, Double outlet right ventricle HP:0001719	NM_001492.4(GDF1):c.681 C > A p.(Cys227*), het mat NM_001492.4(GDF1):c.190 C > T p.(Arg64Cys), het pat	Multiple types of congenital heart defects type 6, right atrial isomerism	P VUS (PM2 PM3)	NA VUS (PM2 PM3)		NA Yes, matching the phenotype
3	32 + 6	Tachycardia HP:0001649, Right atrial enlargement HP:0030718, Hydrops fetalis HP:0001789, Cardiomyopathy HP:0001638	NM_153485.2(NUP155):c.3533 T > A p.(Leu1178Gln), hom bi-parental NM_002225.3(IVD):c.145-4 T > G p.(?), hom bi-parental	Atrial fibrillation type 15 Isovaleric acidemia	VUS (PM2 PP3) VUS (PM2 PP3)	VUS (PM2 PP3) VUS (PM2 PP3)		Yes, matching the phenotype Yes, validation possible and treatable with low burden
4	22 + 3	Agenesis of corpus callosum HP:0001274, Tetralogy of Fallot HP:0001636, Fetal cystic hygroma HP:0010878, Fetal pyelectasis HP:0010945	NM_133647.1(SLC12A6):c.1012 C > T p.(Arg338Cys), hom bi-parental	Agenesis of the corpus callosum with peripheral neuropathy	VUS (PM1 PM2 BP4)	LB (PP3 BS2)	Downgraded because of high population frequency (GnomAD V4.1 allele frequency 0.0004288)	Yes, matching the phenotype
5	21 + 3	Cleft upper lip HP:0000204	NM_004699.3(FAM50A):c.1015 C > T p.(Arg339Cys), hemi mat	X-linked syndromic intellectual disability disorder, Armfield type	VUS (PM2 PP3)	LB (BS3)	Downgraded because of normal in vivo functional validation using zebrafish models	Yes, matching the phenotype
6	23 + 1	Lymphedema HP:0001004, Intrauterine growth retardation HP:0001511 (both resolved at GA 29W0D)	NM_022168.4(IFIH1):c.2159 G > C p.(Arg720Pro), het mat	Aircardi-Goutieres syndrome type 7, Singleton-Merten syndrome type 1	VUS (PS1 PM2 PM5)	VUS (PM2 PM5)		Yes, matching the phenotype
7	35 + 0	Pulmonic stenosis HP:0001642, Double outlet right ventricle HP:0001719, Ventricular septal defect HP:0001629, Transposition of the great arteries HP:0001669, Patent foramen ovale HP:0001655	NM_006767.3(LZTR1):c.2396 A > C p.(Gln799Pro), het pat	Noonan syndrome type 10	VUS (PM1 PM2 PP3)	VUS (PM2)		Yes, matching the phenotype
8	23 + 0	Hypoplastic right heart HP:0010954, Tricuspid atresia HP:0011662, Ventricular septal defect HP:0001629	NM_015093.4(TAB2):c.1727 G > A p.(Arg576His), het mat	Nonsyndromic congenital heart defects type 2	VUS (PM2 PP3 BP4)	VUS (PM2)		Yes, matching the phenotype
9	22 + 3	Ventricular septal defect HP:0001629	NM_001031717.3(CRELD1):c.83 G > A p.(Trp28*), het pat	Partial atrioventricular septal defect with heterotaxy syndrome	VUS (PVS1 PM2 BS4)	LP (PVS1 PM2)	Upgraded because of highly variable expressivity thus BS4 does not apply	Yes, matching the phenotype

Table 1. continued

Case nr	GA at pES result	Phenotype	Variant	Associated OMIM diagnosis	ACMG classification			Report according to flowchart [12] including criteria
					At time of reporting	As applied with currently available data	Details on reclassification	
10	22 + 2	Left ventricular outflow tract obstruction HP:0032092; Coarctation of aorta HP:0001680; Atrioventricular canal defect HP:0006695	NM_001429.4(EP300):c.1517 T > C p.(Met506Thr), het dn	Rubinstein-Taybi syndrome	VUS (PS2)	VUS (PM2 PS2_moderate)		Yes, matching the phenotype
Possible phenotypic consistency of gene-associated diagnoses with fetal phenotypes								
11	18 + 1	Hypoplastic left heart HP:0004383	NM_002471.3(MYH6):c.689 C > T, p.(Ala230Val), het pat	Cardiomyopathy	VUS (PM2 PP3)	VUS (PM2 PP3)		Yes, (partially) matching the phenotype
12	23 + 4	Hypoplastic left heart HP:0004383, Pulmonic stenosis HP:0001642, Coarctation of aorta HP:0001680	NM_002471.3(MYH6):c.1943C>T p.(Thr648Met), het mat	Cardiomyopathy, Atrial septal defect type 3	VUS (PM1 PM2 PP3)	VUS (PM2 PP3)		Yes, (partially) matching the phenotype
13	23 + 4	Multicystic kidney dysplasia HP:0000003	NM_002942.4(ROBO2):c.3236 T > A p.(Val1079Asp), het pat	Vesicoureteral reflux 2	VUS (PM2 BP4)	VUS (PM2)		Yes, (partially) matching the phenotype
14	22 + 1	Ventricular septal defect HP:0001629	NM_003590.5(CUL3):c.1573 C > A p.(Pro525Thr), het dn	Neurodevelopmental disorder with or without autism or seizures	VUS (PS2 PM2 PP3)	VUS (PM2 PP3 PS2_supporting)		Yes, (partially) matching the phenotype
15	29 + 2	Megalencephaly HP:0001355, Enlarged fetal cisterna magna HP:0011427	NM_000264.3(PTCH1):c.271 G > T p.(Gly91Cys), het dn	Holoprosencephaly type 7	VUS (PS2 PM1 PM2 PP3)	VUS PM2 PP3 (PS2_moderate)		Yes, (partially) matching the phenotype
Case reports describing possible consistency of gene-associated diagnoses with fetal phenotypes								
16	22 + 2	Cardiomyopathy HP:0001638, Bilateral talipes equinovarus HP:0001776	NM_007078.2(LDB3):c.59del: p.(Gly20Alafs*41), het mat NM_007078.2(LDB3):c.859+4 A > G: p.(?), het pat	Cardiomyopathy	VUS (PM2) VUS (PM2)	LP (PVS1_strong PM2 PM3) P (PVS1 PM2 PM3)	Pathogenicity validated with functional testing using RNA sequencing and electron microscopy on muscle tissue (PMID 36253531)	Yes, both VUS (partially) matching the phenotype
17	22 + 6	Hypoplasia of the corpus callosum HP:0002079, Widened subarachnoid space HP:0012704, Abnormality of neuronal migration HP:0002269, Intrauterine growth retardation HP:0001511	NM_032110.2(DMRTA2):c.196 C > T p.(Arg66Trp), het pat NM_032110.2(DMRTA2):c.313 C > A p.(Arg105Ser), het mat	NA	VUS (PM1 PM2 PP3) VUS (PM1 PM2 PP3 BP4)	VUS (PM2) VUS (PM2 PP3)		Yes, both VUS (partially) matching the phenotype
18	20 + 1	Dextrocardia HP:0001651, Atrioventricular canal defect HP:0006695, Isomerism HP:0031853 (suspected)	NM_003923.2(FOXH1):c.893 C > T, p.(Thr298Ile), het dn NM_000033.3(ABCD1):c.2051 T > A, p.(Leu684Gln), hemi mat	NA Adrenoleukodystrophy	VUS (PS2 PM2) VUS (PM2 PM5 PP3)	VUS (PM2 PS2_supporting) LP (PS3 PM2 PM5)	 Pathogenicity validated with functional testing on fibroblasts showing significantly decreased expression of ALDP and impaired very-long chain fatty acid metabolism	Yes, (partially) matching the phenotype Yes, validation possible

Table 1. continued

Case nr	GA at pES result	Phenotype	Variant	Associated OMIM diagnosis	ACMG classification			Report according to flowchart [12] including criteria
					At time of reporting	As applied with currently available data	Details on reclassification	
19	33 + 4	Ventriculomegaly HP:0002119	NM_001256012.1(MYH10):c.2856_2860del p.(Glu952Aspfs*4), het dn	NA	VUS (PS2 PM2)	LP (PM2 PVS1_moderate PS2_moderate)	Upgraded because loss-of-function is a known mechanisms of disease and fitting with phenotype	Yes, (partially) matching the phenotype
Increased nuchal translucency as main phenotypic feature								
20	16 + 1	Omphalocele HP:0001539, Increased nuchal translucency HP:0010880, Generalized edema HP:0007430	NM_015107.2(PHF8):c.454+5 G > A p.(?), hemi mat	X-linked syndromic intellectual developmental disorder, Siderius type	VUS (PM2 PP3)	VUS (PM2 PP3)		Yes, validation possible
21	17 + 6	Increased nuchal translucency HP:0010880, Cardiomegaly HP:0001640	NM_020760.1(HECW2):c.3272 A > T p.(Gln1091Leu), het dn	Neurodevelopmental disorder with hypotonia, seizures and absent language	VUS (PS2 PM2 PP3)	VUS (PM2 PP3 PS2_supporting)		No
22	16 + 1	Increased nuchal translucency HP:0010880	NM_007279.2(U2AF2):c.559 T > A p.(Leu187Met), het dn	Developmental delay, dysmorphic facies and brain anomalies	VUS (PS2 PM2)	VUS (PM2 PP2 PS2_supporting BP4)		No
23	16 + 2	Increased nuchal translucency HP:0010880	NM_182641.4(BPTF):c.5468_5470del p.(Lys1823del), het dn	Neurodevelopmental disorder with dysmorphic facies and distal limb anomalies	VUS (PS2 PM2 BP3)	VUS (PM2 PM4 PS2_supporting)		No
Inconsistency of gene-associated diagnoses with fetal phenotype								
24	22 + 2	Fetal hydrothorax HP:0025678, Generalized edema HP:0007430, Abnormality of the diaphragm HP:0000775	NM_003366.2(UQCRC2):c.1255 G > A p.(Val419Met), hom bi-parental	Mitochondrial complex III deficiency, nuclear type 5	VUS (PM1 PM2 BP4)	VUS (no criteria)		Yes, validation possible and treatable with low burden
25	22 + 3	Hypoplasia of the corpus callosum HP:0002079, Postaxial foot polydactyly HP:0001830, Mild fetal ventriculomegaly HP:0010952, Absent septum pellucidum HP:0001331, Hypertelorism HP:0000316	NM_000168.5(GLI3):c.4198dup p.(Asp1400Glyfs*12), het dn	Greig cephalopolysyndactyly syndrome	LP	NA		NA
			NM_004444.4(EPHB4):c.2362 G > A p.(Ala788Thr), het dn	Capillary malformation arterioveneous malformation type 2	VUS (PS2 PM1 PM2 PP3)	VUS (PM1 PM2 PP3)		Yes, treatable with low burden
26	22 + 2	Craniosynostosis HP:0001363, Fetal choroid plexus cysts HP:0011426	NM_014494.4(TNRC6A):c.2402del p.(Gln801Argfs*102), het dn	Familial adult myoclonic epilepsy type 6*	VUS (PS2 PM2)	VUS (PM2)		No
27	16 + 2	Hydrops fetalis HP:0001789, Hypoplastic nasal bone HP:0025707, Abnormality of ductus venosus blood flow HP:0010947	NM_000287.3(PEX6):c.402delC p.(Gly135Aspfs*23), hom bi-parental	Heimler syndrome type 2, peroxisome biogenesis disorder	P	NA		NA
					LP	NA		
			NM_003573.2(LTBP4):c.780+2 T > C p.(?), het pat	Autosomal recessive cutis laxa type 1 C	VUS (PM3 PP3 PP4)	VUS (PM3 BS1)		Yes, treatable with low burden
			NM_003573.2(LTBP4):c.1414 C > T, p.(Arg509Cys), het mat					

Abbreviations; *ACMG* American College for Medical Genetics and Genomics, *(L)B* (likely) benign, dn: de novo, *GA* gestational age, *Hemi* hemizygous, *Het* heterozygous, *Hom* homozygous, *Mat* maternal, *Pat* paternal, *(L)P* (likely) pathogenic, *VUS* variant of unknown significance.

**In addition to gene-associated OMIM diagnosis, also possible developmental delay (patients ascertained through GeneMatcher [20]).*

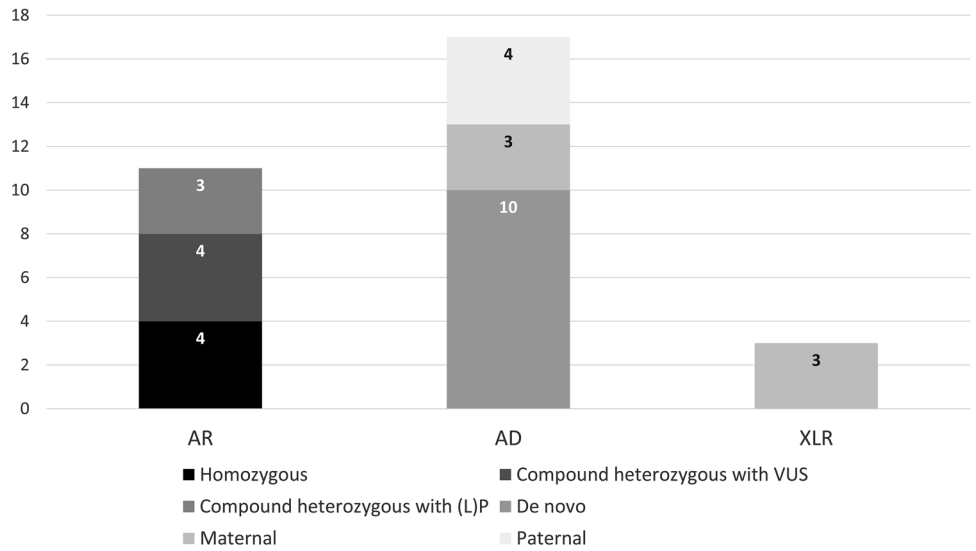

Fig. 1 Inheritance of VUS ($N = 31$ variants). AD autosomal dominant, AR autosomal recessive, (L)P (likely) pathogenic, VUS Variants of Uncertain Significance, XLR x-linked recessive.

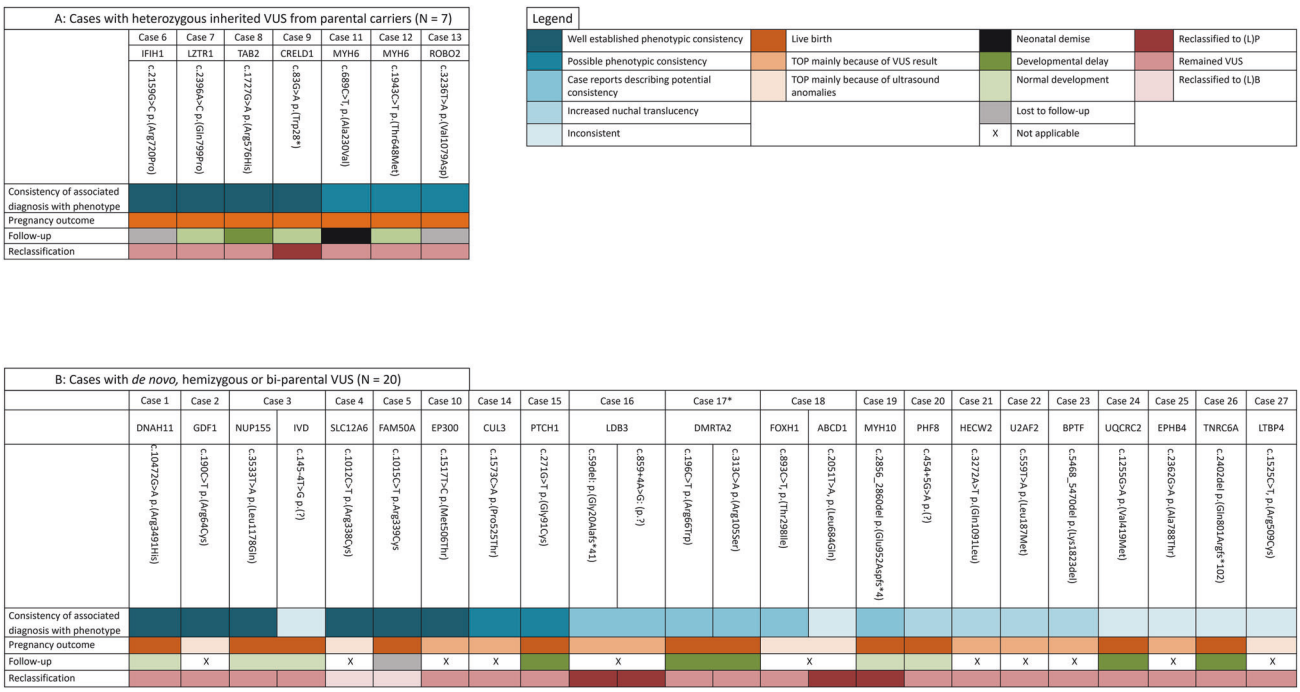

Fig. 2 Pregnancy outcome, follow-up and reclassification of VUS ($N = 27$ cases and 31 variants). A Cases with heterozygous inherited VUS from parental carriers (N = 7 cases), **B** Cases with de novo, hemizygous or bi-parental VUS ($N = 20$ cases). Refer to legend for color-coding of consistency of the gene-associated diagnosis with the fetal phenotype, for pregnancy outcome, for follow-up and for reclassification of VUS using 2015 American College of Medical Genetics and Genomics criteria. *Asterisk* indicates twin pregnancy (both twins affected). (L)B (likely) benign, (L)P likely pathogenic, TOP termination of pregnancy, VUS Variants of Uncertain Significance.

likely truncating VUS in *LDB3* (OMIM 605906). Heterozygous missense variants in this gene are associated with (cardio-) myopathy, but homozygous knock-out mice show a severe form of congenital myopathy [15]. Because of the likely severity of this diagnosis, the parents chose TOP. Postpartum functional validation tests [16] and collection of additional cases supported pathogenicity. Case 17 concerned a monochorionic twin pregnancy in which both fetuses were affected by similar brain anomalies and both carried compound heterozygous variants in *DMRTA2* (OMIM 614804). Bi-allelic variants in this gene have previously been described in a single family with severe prenatal brain abnormalities [17]. At 2.5 years of age both probands suffer from severe microcephaly (-5SD) accompanied by NDD among other issues. In case 18, a de novo variant in *FOXH1* (OMIM 603621) was identified. The ultrasound anomaly was an atrioventricular septal defect with suspected isomerism, consistent with several case reports describing CHD in relation to *FOXH1* [18]. The parents chose TOP mainly based on the ultrasound anomalies. In case 19, a de novo heterozygous VUS in *MYH10* (OMIM 160776) was detected in a fetus presenting with ventriculomegaly. Since this gene has been implicated in brain anomalies including septo-optic dysplasia [2, 19], the proband was tested postpartum but showed no further anomalies. She has a drain and shows normal development at the age of 1 year.

Increased nuchal translucency as main phenotypic feature

In four cases with increased nuchal translucency (NT), VUS were reported in genes related to NDD characterized by intellectual disability with or without congenital anomalies. Since increased NT is a non-specific finding confined to prenatal detection, and many postnatal syndromes do not yet have a well-described prenatal presentation, at the time of reporting it was unknown whether these findings could match the fetal phenotype but all seemed to possibly be relevant information for parents. In case 20, a maternally inherited hemizygous variant was found in *PHF8* (OMIM 300560), which turned out to segregate in the unaffected maternal grandfather after reporting. No additional structural anomalies associated with this possible diagnosis were detected during additional prenatal ultrasound. At age 3 years, the proband has unilateral microtia with hearing loss and mild hypotonia. Functional validation of this variant was inconclusive. In cases 21-23, de novo variants were reported in the genes *HECW2* (OMIM 617245), *U2AF2* (OMIM 191318) and *BPTF* (OMIM 601819), respectively. Parents of all three cases stated they chose TOP based on the uncertain prognosis of the VUS. In case 23, postnatal dysmorphic features fitting the *BPTF* variant were seen, thus solidifying this diagnosis.

Inconsistency of gene-associated diagnoses with fetal phenotypes

VUS in genes thus far unrelated to the fetal phenotype were identified in six cases. These included treatable metabolic disorders in cases 3 and 24, in which biparental variants were identified in *IVD* (OMIM 607036) and *UQCRC2* (OMIM 191329), respectively. The pathogenicity of the *IVD* variant (case 3, in addition to the previously described homozygous VUS in *NUP155*) is yet to be validated since levels of isovaleric acid need to be measured during fever. Case 24 was tested for metabolic acidosis immediately postpartum, which proved negative, thus decreasing the likelihood that the variant is pathogenic. At age 6 the proband shows facial dysmorphic features with mild NDD.

A de novo variant was reported in two cases. In case 25 this involved a variant in *EPHB4* (OMIM 600011), which is associated with arteriovenous malformations, that was absent from population databases and had strong pathogenic prediction scores. Parents of case 25 opted for termination due to the added uncertainty of the *EPHB4* VUS and because possible cerebral arteriovenous malformations could not be reliably assessed prenatally during an extra ultrasound. This variant was reported in addition to a concurrent diagnosis of Greig Cephalopolysyndactyly syndrome (OMIM 175700), which explained the ultrasound anomalies. In case 26, a variant in *TNRC6A* (OMIM 610739) was reported, a gene associated with epilepsy and possible developmental delay (patients ascertained through GeneMatcher [20]). No prenatal validation was possible for either variant. Parents of case 26 were relieved that the detected variant was not associated with lethal or very severe conditions, and thus decided to continue the pregnancy. Delivery was complicated with perinatal asphyxia without any detectable cerebral damage, and the proband shows mild NDD at age 1 year.

In two other cases the reported variants may influence the risk of recurrence. In case 18, a maternally inherited hemizygous variant in *ABCD1* (OMIM 300371) associated with adrenoleukodystrophy (OMIM 300100), was reported in addition to a VUS in *FOXH1*. The *ABCD1* variant showed abnormal enzymatic activity, confirming pathogenicity. In case 27, a maternally inherited variant in *LTBP4* (OMIM 604710) was reported, together with a paternally inherited likely pathogenic *LTBP4* variant that causes cutis laxa. Additionally, bi-parental pathogenic variants associated with Zellweger syndrome (OMIM 614862) were identified in *PEX6* (OMIM 601498), concordant with ultrasound anomalies of fetal hydrops. Together these findings led the parents to opt for TOP.

Variant reclassification and flowchart validation

All variants were reevaluated based on ACMG [13] criteria with additional recommendations and practice guidelines [21–23], current literature, new database entries and postnatal phenotype. Seven variants could be reclassified of which five variants were upgraded to (likely) pathogenic and two downgraded to likely benign. For case 4, updated genome databases (GnomAD version 4.1.0 for GRCh38) now showed an allele frequency greater than expected for the disorder, allowing conclusive reclassification to likely benign. For case 5, in vivo testing using zebrafish models showed no abnormalities [manuscript in preparation]. By contrast, five variants (cases 9, 16, 18 and 19) were upgraded to likely pathogenic based on information from international databases, recent publications or confirmatory functional validation tests.

A flowchart previously published by Diderich et al. [12] offers guidance on when to report highly suspicious prenatal VUS. The most important factor in this chart is whether a variant is found in a gene with an associated phenotype that matches the fetal phenotype. Flowchart use was suitable for the first three categories, (i) a well-established phenotypic consistency ($N = 10$ cases), (ii) a possible phenotypic consistency ($N = 5$ cases) or (iii) single case reports ($N = 4$ cases). Of the six reported variants that had no gene-associated consistency with the detected fetal phenotypes, treatment (cases 3, 24, 25 and 27) and/or rapid functional validation (cases 3, 18 and 24) was possible in five cases, which justifies reporting of these variants to parents according to the flowchart. The flowchart does not advise reporting VUS in cases where increased nuchal translucency was the main phenotypic feature ($N = 4$). When strictly applying flowchart criteria retrospectively, only one of those four cases qualified for reporting (case 20; due to validation by extended segregation analysis). Based on flowchart criteria, four variants overall would not have been reported (cases 21–23 and 26).

DISCUSSION

With the increasingly routine application of ES during pregnancy, the issue of whether to report VUS or not is becoming progressively relevant. Our study shows that guidelines for reporting VUS prenatally are not easy to establish due to incompleteness of fetal phenotypes and limited time to conduct confirmatory (functional) testing. Although we do not recommend routine VUS disclosure, both academic hospitals encountered about three cases per year, in which suspicious VUS were reported prenatally after careful multidisciplinary consideration. Family segregation, functional validation tests and/or postnatal screening were done when possible, to minimize the burden of uncertainty. Although stringent selection criteria were applied and many follow-up tests were performed, only a minority of reported VUS could be re-classified to (likely) pathogenic which is in line with previous literature on CNV-analysis [14]. Based on currently available resources, five variants in four cases could be upgraded to (likely) pathogenic (16%). Postnatal functional testing allowed upgraded reclassification of the hemizygous variant in case 16 and both compound heterozygous variants in case 18. In case 9 and 19, updated international databases (e.g. ClinVar [24] and ClinGen [25]) and recently published literature allowed upgraded classification. Parents opted for TOP in two of these cases, once because of the added uncertainty of the VUS (case 16) and once mainly because of the severity of the ultrasound anomalies (case 18). Contrarily, two variants were downgraded to (likely) benign (6%) because of updated population databases showing an allele frequency greater than was expected for the disorder (case 4) or because in vivo functional testing showed no abnormalities (case 5). Parents of case 4 opted for termination due to the severity of the ultrasound anomalies. The results of the negative functional validation testing in case 5 were available before the Dutch legal

limit of TOP (24 weeks of gestational age) and thus parents felt reassured to continue the pregnancy.

We also re-evaluated a previously published flowchart aimed at aiding decision making on reporting of prenatal VUS [12]. Reporting the aforementioned VUS was supported by this flowchart in most cases. In four cases, we felt we had valid arguments at the time of detection to deviate from the flowchart. All four variants were de novo and the concerning genes were associated with developmental delay and intellectual disability syndromes. Ultrasound anomalies of these cases were nonspecific (increased nuchal translucency in cases 21–23 and craniosynostosis in case 26) and validation of variants was not possible. Many known genetic syndromes do not (yet) have well-defined prenatal phenotypes, some phenotypic features, such as NDD, are impossible to establish prenatally, and many phenotypes may not have fully developed complicating the assessment of potential neonatal dysmorphic features [26]. Because of this, it can be difficult to confidently discard a potential link between a variant and the detected ultrasound anomalies. This means the ACMG [13] 'Pathogenic Strong 2' (de novo) criterion, which is only valid when the patient's phenotype matches a gene-disease association with reasonable specificity [27], is difficult to apply in a prenatal setting and might sometimes be applied more leniently than postnatally by some laboratories. We suggest to specifically explicate the application and the potential consequences of different interpretations of this criterion for the prenatal setting, so variant classification can be considered with all available evidence when application of this criterion would lead to upgrading.

Although we do not condone reporting VUS in genes associated with adult-onset conditions as incidental findings, the term 'incidental finding' did not encompass the uncertainty of inconsistent gene-associated diagnoses with fetal phenotypes. Since de novo variants are more difficult to interpret by default, we recommend discussing de novo variants in genes associated with pediatric-onset of disease in multidisciplinary team meetings between at least laboratory specialists and clinical geneticists to assess potential pathogenicity, causality and whether it would be desirable to report the variant based on case-specific characteristics such as parental preferences and perspectives and severity of the ultrasound anomalies. Whether or not to actually report the variant should thus be assessed on a case-by-case basis. Even though the ACMG criteria improve the process of variant classification, it remains sensitive to subjective interpretations, because it relies on criteria that are not always quantifiable. The probability of pathogenicity of suspicious VUS might in some cases not even be much lower compared to variants that barely meet the criteria to be classified as likely pathogenic.

Ethical considerations

Prospective parents generally want as much information as possible about their unborn child [10, 28–30] but feel ill-prepared when receiving uncertain results, leading to uncertainty and anxiety. It is a difficult balance between adopting a paternalistic approach that seeks to avoid harm by withholding information that parents may not be equipped to deal with versus an approach that emphasizes reproductive autonomy through full disclosure. However, the question remains whether reproductive autonomy actually increases when uncertainty is increased. A request model – in which VUS are disclosed only upon active request by parents after detailed pretest counseling [31] – is proposed by healthcare professionals working in prenatal genetics [11, 32]. Adequate pretest counseling of parents is therefore imperative, and should discuss not only possible DNA outcomes but also parental preferences regarding the extent of shared information as well as their ability to cope with uncertainty.

Another important factor is whether a variant is '*actionable*' [33], meaning that potential consequences 'can be acted upon' by adjusting pre- or postnatal clinical management. A list of 'actionable fetal findings' was recently proposed [34], in which the authors defined 'actionable' as disorders with available or emerging fetal therapies, and those with improved outcomes following clinical detection in the first week of life (such as case 3 in our cohort). Although controversial, in the prenatal setting one could argue that a choice for termination may be considered 'actionable'. We indeed observed that information concerning a VUS can be of added value to parents who desire comprehensive information on their unborn child's prognosis. However, anxiety levels resulting from uncertain results were not objectively assessed in this study. The considerations of reporting prenatal VUS should be carefully weighed in each case, especially because of the potential harm for a couple that took a decision based on an uncertain pES result, if this variant is subsequently reclassified to (likely) benign. Generally however, decisions regarding TOP were highly personal and dependent on each parents' specific situation. The added value of information on a 'hot' VUS for those considering TOP varies and is influenced by the type of variant, the associated phenotype and the degree of specificity of the match with the fetal phenotype.

Furthermore, the implications of reporting VUS on subsequent pregnancies should be taken into account. If pathogenicity is later proven, VUS may enable reproductive options such as prenatal invasive genetic testing or preimplantation genetic testing (such as in cases 16 and 18). However, there is no consensus on the implications of variants that could not be reclassified. Practice even differs between the two participating centers where one center does facilitate prenatal testing in future pregnancies for the VUS after careful counseling. After all, when a VUS is suspicious enough to report during pregnancy and for parents to opt for termination in some cases, it seems consistent to allow prenatal testing in subsequent pregnancies after careful counseling unless the variant is reclassified to likely benign or became less suspicious in light of new information acquired through follow-up. In the other center, prenatal invasive testing of VUS is not an option, which is in line with common practice for postnatally identified VUS. This dilemma underlines the importance of postmortem physical examination after TOP to gain additional information that might aid reclassification of the variant.

CONCLUSION

To conclude, our experiences of reporting highly suspicious VUS in a prenatal setting highlighted challenges regarding variant classification, and raised ethical issues concerning prospective parents' choices and the role of healthcare professionals in deciding on what to report. Although we do not recommend routine disclosure, our data suggest that prenatal reporting of VUS can be valuable in exceptional instances, but individual assessment of each VUS case is imperative and multidisciplinary meetings should be an integral part of prenatal VUS management. Stringent selection was applied and only a minority of VUS was reclassified as (likely) pathogenic (16%). The burden of uncertainty should be balanced with the likelihood of reclassification of variants in the near future, also taking into account parental specific preferences as discussed during pretest counseling. We therefore advocate that both parents' and clinicians' perspectives regarding VUS should be included in future research and that the clinical follow-up of live born children with prenatally-detected VUS as well as postmortem examination following TOP should be intensified to improve current guidelines.

DATA AVAILABILITY

Data relevant to this study are available from the corresponding author upon request.

REFERENCES

1. Lord J, McMullan DJ, Eberhardt RY, Rinck G, Hamilton SJ, Quinlan-Jones E, et al. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study. Lancet. 2019;393:747–57.
2. Petrovski S, Aggarwal V, Giordano JL, Stosic M, Wou K, Bier L, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet. 2019;393:758–67.
3. Diderich KEM, Bruggenwirth HT, Joosten M, Thurik F, Mijalkovic J, Polak M, et al. The high diagnostic yield of prenatal exome sequencing followed by 3400 gene panel analysis in 629 ongoing pregnancies with ultrasound anomalies. Prenat Diagn. 2024;44:1444–50.
4. de Koning MA, Haak MC, Adama van Scheltema PN, Peeters-Scholte C, Koopmann TT, Nibbeling EAR, et al. From diagnostic yield to clinical impact: a pilot study on the implementation of prenatal exome sequencing in routine care. Genet Med. 2019;21:2303–10.
5. Mastromoro G, Guadagnolo D, Khaleghi Hashemian N, Marchionni E, Traversa A, Pizzuti A. Molecular approaches in fetal malformations, dynamic anomalies and soft markers: diagnostic rates and challenges-systematic review of the literature and meta-analysis. Diagnostics. 2022;23;12:575.
6. Van den Veyver IB, Chandler N, Wilkins-Haug LE, Wapner RJ, Chitty LS. International Society for Prenatal Diagnosis Updated Position Statement on the use of genome-wide sequencing for prenatal diagnosis. Prenat Diagn. 2022;42:796–803.
7. Committee Opinion No. 693: Counseling About Genetic Testing and Communication of Genetic Test Results. Obstet Gynecol. 2017;129:e96-e101.
8. Committee Opinion No.682: Microarrays and next-generation sequencing technology: the use of advanced genetic diagnostic tools in obstetrics and gynecology. Obstet Gynecol. 2016;128:e262-e268.
9. Klapwijk JE, Srebniak MI, Go A, Govaerts LCP, Lewis C, Hammond J, et al. How to deal with uncertainty in prenatal genomics: A systematic review of guidelines and policies. Clin Genet. 2021;100:647–58.
10. Harding E, Hammond J, Chitty LS, Hill M, Lewis C. Couples experiences of receiving uncertain results following prenatal microarray or exome sequencing: a mixed-methods systematic review. Prenat Diagn. 2020;40:1028–39.
11. Lewis C, Hammond J, Klapwijk JE, Harding E, Lou S, Vogel I, et al. Dealing with uncertain results from chromosomal microarray and exome sequencing in the prenatal setting: An international cross-sectional study with healthcare professionals. Prenat Diagn. 2021;41:720–32.
12. Diderich KEM, Klapwijk JE, van der Schoot V, van den Born M, Wilke M, Joosten M, et al. The role of a multidisciplinary team in managing variants of uncertain clinical significance in prenatal genetic diagnosis. Eur J Med Genet. 2023;66:104844.
13. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. 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;17:405–24.
14. Shi P, Liang H, Hou Y, Chen D, Ren H, Wang C, et al. The uncertainty of copy number variants: pregnancy decisions and clinical follow-up. Am J Obstet Gynecol. 2023;229:170.e1–e8.
15. Zhou Q, Chu PH, Huang C, Cheng CF, Martone ME, Knoll G, et al. Ablation of Cypher, a PDZ-LIM domain Z-line protein, causes a severe form of congenital myopathy. J Cell Biol. 2001;155:605–12.
16. Koopmann TT, Jamshidi Y, Naghibi-Sistani M, van der Klift HM, Birjandi H, Al-Hassnan Z, et al. Biallelic loss of LDB3 leads to a lethal pediatric dilated cardiomyopathy. Eur J Hum Genet. 2023;31:97–104.
17. Urquhart JE, Beaman G, Byers H, Roberts NA, Chervinsky E, O'Sullivan J, et al. DMRTA2 (DMRT5) is mutated in a novel cortical brain malformation. Clin Genet. 2016;89:724–7.
18. Wei W, Li B, Li F, Sun K, Jiang X, Xu R. Identification of FOXH1 mutations in patients with sporadic conotruncal heart defect. Clin Genet. 2020;97:576–85.
19. Tuzovic L, Yu L, Zeng W, Li X, Lu H, Lu HM, et al. A human de novo mutation in MYH10 phenocopies the loss of function mutation in mice. Rare Dis. 2013;1:e26144.
20. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat. 2015;36:928–30.
21. Durkie M, Cassidy E, Berry I, Owens M, Turnbull C, Scott RH, et al. ACGS Best Practice Guidelines for Variant Classification in Rare Disease: Association for Clinical Genomic Science; 2024 [updated 20 February 2024. Available from: https://www.acgs.uk.com/media/11631/uk-practice-guidelines-for-variant-classification-v4-01-2020.pdf.
22. Walker LC, Hoya M, Wiggins GAR, Lindy A, Vincent LM, Parsons MT, et al. Using the ACMG/AMP framework to capture evidence related to predicted and observed impact on splicing: Recommendations from the ClinGen SVI Splicing Subgroup. Am J Hum Genet. 2023;110:1046–67.
23. Abou Tayoun AN, Pesaran T, DiStefano MT, Oza A, Rehm HL, Biesecker LG, et al. Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. Hum Mutat. 2018;39:1517–24.
24. Landrum MJ, Chitipiralla S, Kaur K, Brown G, Chen C, Hart J, et al. ClinVar: updates to support classifications of both germline and somatic variants. Nucleic Acids Res. 2025;53:D1313–d21.
25. Rehm HL, Berg JS, Brooks LD, Bustamante CD, Evans JP, Landrum MJ, et al. ClinGen-tHE CLINICAL GENOME REsource. N Engl J Med. 2015;372:2235–42.
26. Basel-Salmon L, Sukenik-Halevy R. Challenges in variant interpretation in prenatal exome sequencing. Eur J Med Genet. 2022;65:104410.
27. Group CSVIW. SVI Recommendation for De Novo Criteria (PS2 & PM6) - Version 1.1 2021 [Available from: https://clinicalgenome.org/site/assets/files/3461/svi_proposal_for_de_novo_criteria_v1_1.pdf.
28. Hammond J, Klapwijk JE, Riedijk S, Lou S, Ormond KE, Vogel I, et al. Assessing women's preferences towards tests that may reveal uncertain results from prenatal genomic testing: Development of attributes for a discrete choice experiment, using a mixed-methods design. PLoS ONE. 2022;17:e0261898.
29. Bernhardt BA, Soucier D, Hanson K, Savage MS, Jackson L, Wapner RJ. Women's experiences receiving abnormal prenatal chromosomal microarray testing results. Genet Med. 2013;15:139–45.
30. Talati AN, Gilmore KL, Hardisty EE, Lyerly AD, Rini C, Vora NL. Parental motivations for and adaptation to trio-exome sequencing in a prospective prenatal testing cohort: Beyond the diagnosis. Prenat Diagn. 2022;42:775–82.
31. Watts G, Newson AJ. To offer or request? Disclosing variants of uncertain significance in prenatal testing. Bioethics. 2021;35:900–9.
32. Hui L, Szepe E, Halliday J, Lewis C. Maternity health care professionals' views and experiences of fetal genomic uncertainty: a review. Prenat Diagn. 2020;40:652–60.
33. Horn R, Parker M. Opening Pandora's box?: ethical issues in prenatal whole genome and exome sequencing. Prenat Diagn. 2018;38:20–5.
34. Cohen JL, Duyzend M, Adelson SM, Yeo J, Fleming M, Ganetzky R, et al. Advancing precision care in pregnancy through an actionable fetal findings list. medRxiv. 2024:2024.09.26.24314442.

AUTHOR CONTRIBUTIONS

The project was conceptualized by MK and GS with input and feedback from MSue and MSreb. Data curation was performed by MK, DH, MH, MSreb, KD and HB. Initial drafting of the manuscript was done by MK, under supervision of MSue. All authors were involved in drafting, editing and reviewing the final version of the manuscript.

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COMPETING INTERESTS

The authors declare no competing interest.

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