Diagnosis of fetal structural abnormalities using exome sequencing: a single centre study

Helen Savage[1], Louisa Ive[1], Vijaya Ramachandran[1], Laura Reed[1], Andrea Haworth[1], Rand Dubis[1], Eva Serra[1], John Short[2], Nick Lench[1], Basky Thilaganathan[3], Sahar Mansour[2], Esther Dempsey[2], Tessa Homfray[2] and Suzanne Drury[1]

1. Congenica Ltd, Biodata Innovation Centre, Wellcome Genome Campus, Hinxton, CB10 1DR
2. South West Thames Regional Genetics, St George's Hospital, London, SW17 0QT
3. Fetal Medicine Unit, St George's Hospital, London, SW17 0QT

Introduction

Approximately 2-5% of pregnancies display fetal development anomalies on a routine ultrasound (US) scan, however current testing demonstrates a genetic etiology in only 40% of cases.

A review by Best *et al* (2018) demonstrated the utility of Exome Sequencing (ES) for improved diagnosis in fetuses with structural anomalies; reported diagnostic rates were between 6.2-80%. Currently, ES is a second-line test, *after* aCGH; there is therefore potential diagnostic benefit in providing a single comprehensive, first-line test for Single Nucleotide Variants (SNVs), indels and Copy Number Variants (CNVs).

Objectives

1. Undertake ES to provide a molecular diagnosis for cases of unexplained fetal anomalies.
2. Implement an improved exome assay for SNV, indel and CNV detection.

Method

Results

Diagnostic rate. A diagnosis was established in 40/104 cases. In 14/40 of diagnoses (35%) the causal variant had not been described previously.

Family structure	#	Diagnostic rate	Causative SNVs	Causative CNVs
Singleton	30	60%	*ALPL, BICD2, COL1A2, FGFR2, GREB1L, KCNJ2, LZTR1, MYO7A, NIPBL, NR2F2, PAFAH1B1, PTPN11, RAF1, RMRP, SLC17A5, SLC26A2*	
Duo	3	67%	*GNPTAB, UBE2A*	
Trio	67	28%	*CENJP, CHD7, COL1A1, EVC2, FAM111A, FGFR3, FOXC1, KMT2D, OFD1, PIEZO1, POMGNT1, PTPN11, SLC6A9, SOS1, TUBA1A*	Paternal 1p21 deletion, including *RBM8A;* Maternal 70kb deletion, including *SUMF1*
Quad	4	25%	*P3H1*	
Total	**104**	**38%**		

Table 1: Diagnostic rate observed across family structures

Variants of uncertain significance (VUS) and uncertain clinical significance.

- In 7% (7/104) of cases, variants reported as VUS or VUCS were determined to be the cause of fetal anomaly on clinical review.
- Includes VUS and likely pathogenic/pathogenic VUCS variants which did not fit the original suspected clinical diagnosis.

Diagnosis by phenotype. In contrast to published unselected cohorts (Lord et al 2019; Petrovski et al 2019), a higher diagnostic yield was obtained by careful patient ascertainment, involving fetal medicine and clinical genetics. Of particular interest, 32/104 fetuses in this cohort had an edematous phenotype (raised NT or hydrops); of these, 56% (18/32) had a confirmed molecular diagnosis.

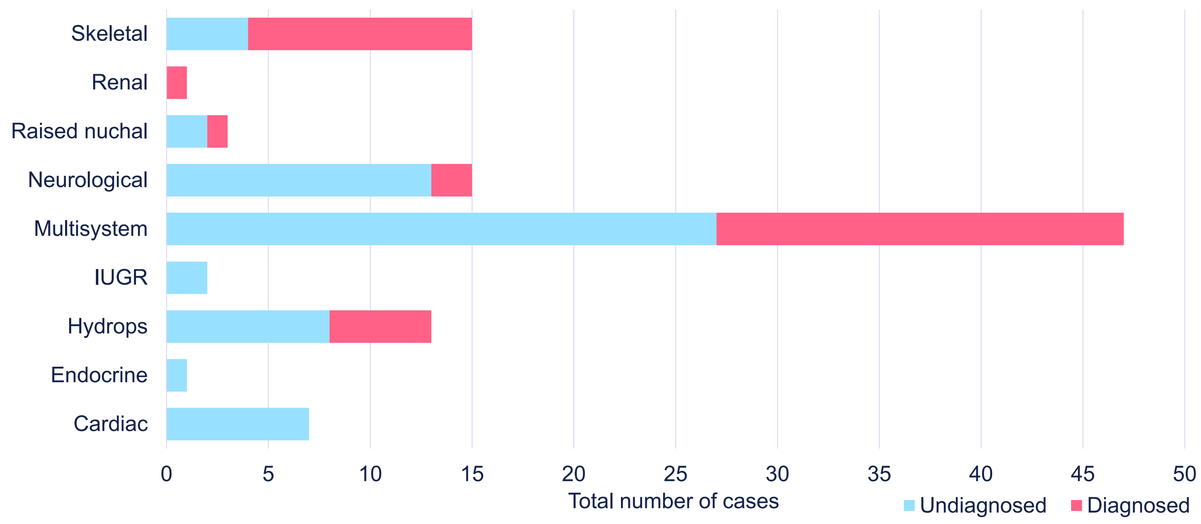

Figure 1: Number of diagnosed versus undiagnosed by phenotype. "Multisystem" describes fetuses with complex phenotypes affecting more than one major bodily system.

	VUCS Case 1 - Suspected Osteogenesis Imperfecta	CNV case 1 – TAR syndrome	CNV case 2 – Multiple Sulfatase Deficiency
Case background	Short long bones and fractures observed at 20 week scan. Marginally abnormal shaped chest	Short humerus, absent radius and ulna on the right arm, bilateral wrist flexion. Lower limbs appear normal. History of early miscarriages. Trio analysis performed.	Hydrops, increased NT, echogenic left kidney and absent/pelvic right kidney, echogenic bowel, right ventricular brightness/hypertrophy.
Variants identified	Compound heterozygous variants in the *ALPL* gene	Compound heterozygous variants affecting *RBM8A* detected. Maternally-inherited SNV in the 5' UTR of *RBM8A* and a paternally-inherited inherited deletion of 1p21, including *RBM8A*.	Compound heterozygous variants affecting *SUMF1.* Maternally-inherited 70kb deletion, including *SUMF1* and a paternally-inherited frameshift within *SUMF1*.
Postnatal phenotype described	Causal variants in *ALPL* are associated with Hypophosphatasia, which presents postnatally with defective mineralization of bone and/or teeth	Causal variants in RBM8A are associated with Thrombocytopenia-absent radius syndrome. This disorder is characterized by thrombocytopenia, congenital aplasia of the radii, ulnar aplasia and short stature.	Causal variants in *SUMF1* are associated with Multiple Sulfatase Deficiency. This disorder is characterized by leukodystrophy, seizures, developmental delay, slow growth, ichthyosis, hypertrichosis and skeletal anomalies.
Prenatal phenotype described	Prenatal disease mimics osteogenesis imperfecta and often appears more serious than manifests in the postnatal period	Prenatal presentation includes bilateral radial hypoplasia/agenesis, with or without humeral shortness, and presence of thumbs on both hands.	Prenatal presentation of this disorder is poorly described however hydrops fetalis and ascites have been reported in association with *SUMF1*.
Outcome/ Impact	Treatment available for paediatric-onset hypophosphatasia with Asfotase alfa. Father has low Alkaline Phosphatase (ALP). Maternal ALP inaccurate in pregnancy and not tested.	No curative treatment is available for TAR syndrome, however early diagnosis allows thrombocytopenia to be monitored and severity reduced.	While no treatment is available for this severe, congenital form of the disease, the couple have access to prenatal diagnosis in any future pregnancy, enabling prenatal management.

Table 2: Examples of a VUCS and contributing CNVs determined to be the cause of fetal anomaly upon clinical review

Conclusion

- Careful patient ascertainment yields a high diagnosis (38%).
- Combining SNV and CNV analysis in a single test, such as ExomeCG, increases the diagnostic yield and reduces the time taken to report results during pregnancy.
- Prenatal diagnosis alters:
  - prenatal management, postnatal treatment, management of future pregnancies
- Return of variants of uncertain clinical significance to clinical geneticists with expertise in the prenatal setting can yield additional diagnoses, emphasising the need for multidisciplinary team approaches.
- A molecular diagnosis is not always the primary clinical diagnosis, due to lack of prenatal presentation phenotype data.

info@congenica.com

congenica.com