Enabling genomic medicine

The Impact of Molecular Diagnosis of Fetal Structural Anomalies Using Exome Sequencing

Rand Dubis[1], Suzanne Drury[1], Andrea Haworth[1], Nick Lench[1], Helen Savage[1], Eva Serra[1], John Filby[1], Natalie Trump[1], Louisa Ive[1], Basky Thilaganathan[3], John Short[2], Charlene Crosby[2], Sahar Mansour[2], Tessa Homfray[2] and Esther Dempsey[2]

[1]Congenica, Cambridge, UK, [2]St. George's University of London, London, UK, [3]Fetal Medicine Unit, St. George's Hospital, London, UK

Introduction

Fetal anomalies are found in 2-5% of pregnancies upon Ultrasound Screening (USS). A genetic aetiology is identified in ~40% of these cases using current testing strategies, leaving the majority undiagnosed.
Prenatal diagnosis provides information for prenatal, perinatal and postnatal management and treatment. It also provides an accurate assessment of recurrence in future pregnancies.
Exome Sequencing (ES) has been shown to improve diagnostic rates in fetuses with structural anomalies. A review by Best *et al* (2018) has demonstrated diagnostic rates between 6.2% - 80%. As ES is typically offered after chromosomal microarray, there is a potential benefit in a single comprehensive test for single nucleotide variants (SNVs), indels and copy number variants (CNVs).

Objectives

1. To provide a genetic diagnosis for cases displaying fetal anomalies detected by USS using ES, with phenotypes derived from USS and/or post-mortem analysis.
2. To implement an improved ES assay for the analysis of SNVs, indels and CNVs.
3. To assess the clinical impact of prenatal ES on the management of pregnancy and treatment of neonates.

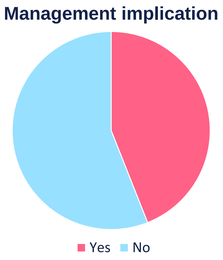
Results

Through ES, a positive diagnosis was achieved in 55 out of 163 fetuses referred (34%). Of 75 cases assessed for CNVs, 2 had pathogenic variants (3%).

ACTG2	*ASPM*	*B9D1*	*BICD2*	*CENPJ*	*CHD7*	*COL1A1*
COLA12	*DHCR1*	*EVC*	*EVC2*	*FAM111A*	*FGFR3*	*FREM2*
GNPTAB	*KCNQ1*	*LZTR1*	*MLH1*	*MYH3 (2)*	*MYO7A*	*NIPBL*
NR2F2 (2)	*OFD1*	*P3H1 (2)*	*PAFAH1B1*	*PEX19*	*PIEZO1 (2)*	*POMGNT1*
PTPN11 (5)	*RAF1 (3)*	*RBM8A*	*RMRP*	*SLC17A5*	*SLC26A2 (2)*	*SLC6A9*
SOS1 (2)	*SUMF1*	*TCF12*	*TSC1*	*TTN*	*TUBA1A (2)*	*UBE2A*

Table 1: The 42 diagnostic genes identified in the positive prenatal cases. (n) = number of diagnoses if greater than 1.

A molecular diagnosis by prenatal exome sequencing impacted management in 8/18 (44%) ongoing pregnancies reviewed for management implications.



Negative molecular diagnoses were also informative for management (see example case studies below).

Methods

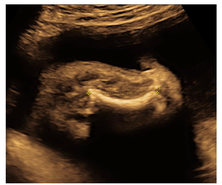
Cases of 163 fetal anomalies detected on US were referred to Clinical Genetics at St George's Hospital, London. Fetal DNA was extracted from CVS, amniotic fluid, fetal blood or post-mortem fetal tissue. Maternal cell contamination was exclued where relevant. DNA was enriched using Agilent SureSelect Clinical Research Exome V2 (CRE V2) or Nonacus ExomeCG and sequenced on Illumina NextSeq 500 or NovaSeq. Secondary and tertiary analysis of DNA sequences and review of SNVs and CNVs was undertaken using the Congenica clinical decision platform. Analysis of CNVs was performed only on DNA samples enriched with Nonacus ExomeCG. In 52 ongoing pregnancies, the impact of diagnosis on management was reviewed (PMID: 32981126).

Discussion

This study illustrates the utility of combined CNV and SNV calling in a rapid testing scenario and the impact of prenatal ES on pregnancy management, postnatal therapy and surveillance, reproductive decision making and additional implications for the wider family. Molecular diagnosis was not always concordant with the primary clinical diagnosis, emphasising both the challenge of reduced phenotype details in prenatal cases and the importance of expanding prenatal genotype-phenotype correlations.

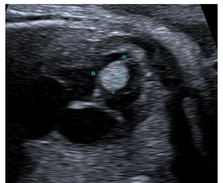
Case Studies – Impact of Diagnosis

Case 1 – Suspected Osteogenesis Imperfecta



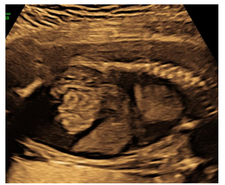
- Short long bones and fractures observed at 20-week scan
- Compound heterozygous variants in the *ALPL* gene
- Treatment available for paediatric-onset hypophosphatasia with Asfotase alfa. Father has low Alkaline Phosphatase (ALP). Maternal ALP inaccurate in pregnancy and not tested
- Parents provided with access to rheumatology and dental services for surveillance as carriers can manifest symptoms

Case 2 – Ventricular Septal Tumour



- Isolated ventricular septal tumour upon 23-week ultrasound scan
- Targeted analysis of *TSC1* and *TSC2* genes did not detect any causal variants and pregnancy continued
- *TSC1* and *TSC2* account for 85% of Tuberous Sclerosis Complex (TSC) cases
- The child was born healthy and will continue to be monitored
- Low recurrence risk in future pregnancies (<1%)

Case 3 – Comprehensive Workflow



- Fetal hydrops, echogenic bowel and renal abnormalities identified at a 21-week US
- Compound heterozygous variants affecting *SUMF1* gene detected: CNV and SNV
- The gene is associated with multiple sulfatase deficiency
- No treatment available
- The couple will have access to prenatal diagnosis in future pregnancies