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Non-invasive prenatal solutions for multiple single gene disorders in a single test – Improved Next Generation Sequencing for Ultrasound Abnormalities (INGENIOUS)

Suzanne Drury[1], Rand Dubis[1], Tim Rayner[1], John Short[2], Rachel Dunn[2], Tessa Homfray[2], Sahar Mansour[2], Lisa Canclini[3], Stephanie Allen[4], Nick Lench[1], Gavin Guy[3] and Basky Thilaganathan[3]

[1] Congenica Ltd, Wellcome Genome Campus, Hinxton, CB10 1DR; [2] Clinical Genetics, St George's University Hospitals NHS Foundation Trust, London SW17 0RE; [3] Fetal Medicine Unit, St George's University Hospitals NHS Foundation Trust, London SW17 0RE [4] West Midlands Regional Genetics Laboratory, Birmingham Women's Hospital, Birmingham, B15 2TG

Introduction

- Fetal anomalies are identified in ~3% of pregnancies and are responsible for ~20% of perinatal deaths.
- Approximately 60% of causative mutations in unselected fetal anomaly case series are *de novo* (PMID: 30712880; 30712878).
- *De novo* mutation rate increases with parental age (PMID: 22914163, 28135719).
- Invasive fetal sampling is currently the only way to comprehensively test fetal genomic material for single gene disorders and is restricted to over 11 weeks gestation.
- Existing non-invasive methods for single gene disorders are targeted to a handful of genes at a time when prenatal phenotype-genotype knowledge is expanding.
- Prenatal molecular diagnosis can inform pregnancy management, (PMID:32981126)
- Non-invasive testing for aneuploidy using cell free DNA (cfDNA) is now widely available from 9 weeks gestation.

Objective

To develop a comprehensive assay, analytical and reporting workflow for non-invasive detection of de novo mutations associated with fetal anomalies.

Methods

Couples with fetal anomaly detected on ultrasound were recruited to study (REC 18/WA/0221).

Maternal, paternal and fetal gDNA and maternal cfDNA (quads) from the same pregnancy was enriched for ~2000 genes associated with fetal anomalies & severe early onset disorders using Cell3™ Target custom assay (Nonacus, UK).

Libraries were sequenced using Illumina NextSeq500 or NovaSeq. Sequencing data was aligned using DRAGEN (v3.2.5) and deduplicated using unique molecular indexes.

De novo variants in fetal gDNA were identified by DeNovoGear, Octopus and Mendelian. Variants with a VEP consequence of transcript ablation through to splice region variant were included as the gold standard for comparison.

Putative *de novo* variants in cfDNA, were identified by a custom secondary analysis pipeline including incorporation of fetal fraction, quality metrics and a dynamic error calling algorithm. Data from 73 quads was obtained.

Results

- Gestation range: 10+6 to 36+4
- Median fetal fraction 12% (3-38%)
- Regardless of variant consequence, 30/43 true *de novo* variants were identified in the cfDNA sample
- After VEP consequence filtering, 16/17 (94%) true *de novo* variants were identified in the cfDNA sample
- 6/6 (100%) true pathogenic *de novo* variants were identified in the cfDNA sample (table 1).
- Sensitivity and NPV were high, specificity and PPV were low (table 2).
- A median of 1 putative *de novo* variant per case was identified (mode 0, range 0-8).

Gestation (weeks)	Fetal fraction	Gene	Condition	REF/ALT
15	13%	*RAF1*	Noonan syndrome 5	310/25
28	23%	*FGFR3*	Achrondoplasia	427/56
14	16%	*SOS1*	Noonan syndrome 4	675/38
21	13%	*COL1A1*	Osteogenesis imperfecta	627/40
n/a	22%	*NRAS*	Noonan syndrome 6	269/25
n/a	16%	*NRAS*	Noonan syndrome 6	167/17

Table 1: Summary of cases with molecular diagnoses. All disease causing variants were identified in the non-invasive sample.

Metric	Combined
True positive (TP)	14
False positive (FP)	35
False negative (FN)	2
True negative (TN)	21
Analytical sensitivity (TP/(TP+FN))	88%
Analytical specificity (TN/(TN+FP))	38%
Analytical positive predictive value (TP/(TP+FP))	29%
Analytical negative predictive value (TN/(TN+FN))	91%

Table 2: Analytical validation results.

Discussion

- This was a proof-of-concept study focussed on technical feasibility. Analysis was not restricted to phenotype relevant genes, which is expected to further reduce the number of variants for review.
- The INGENIOUS comprehensive gene panel and secondary analysis pipeline can be used to detect causative *de novo* variants in cfDNA. This is the most extensive non-invasive prenatal panel presented to date.
- Further work is ongoing to improve sensitivity and increase support for inherited dominant and recessive conditions.

info@congenica.com
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