

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

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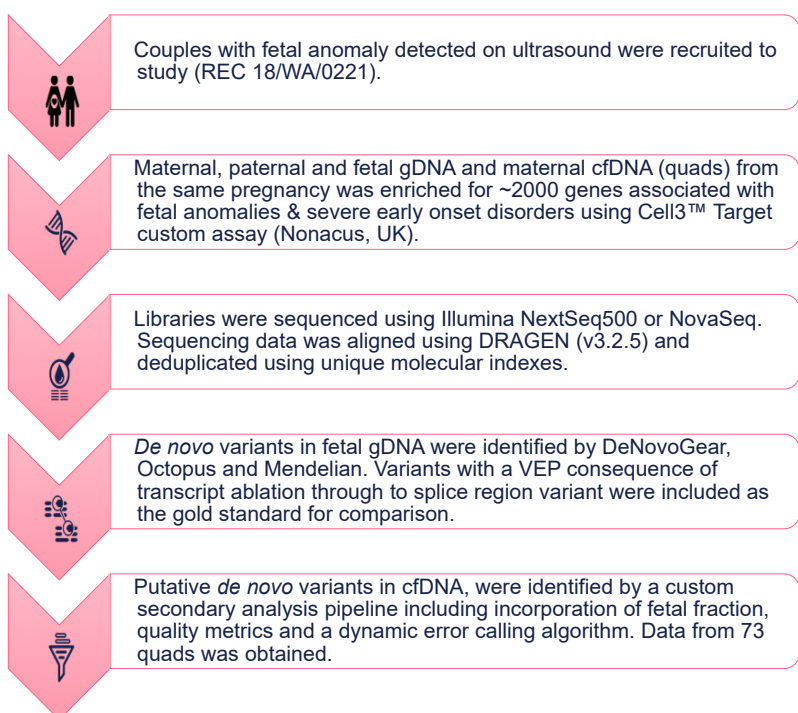
Introduction

- ❖ 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



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 | Achondroplasia | 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.