Towards Improved Next Generation Sequencing for Ultrasound Abnormalities (INGENIOUS) using cell-free DNA

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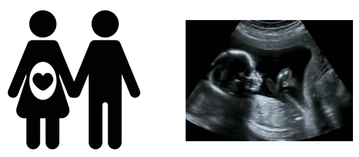
Introduction

- Fetal anomalies are identified in ~2-5% of pregnancies and are responsible for ~20% of perinatal deaths.
- *De novo* mutations (DNM) cannot be detected by testing parental samples.
- 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).
- Non-invasive testing for aneuploidy using cell free DNA (cfDNA) is now widely available from 9 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.
- Fetal DNA is present in the minority of cfDNA and therefore must be distinguished from background noise.
- 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.

Objective

- To develop a non-invasive test for the early molecular diagnosis of fetal anomalies

Method



1. Couples with fetal anomaly detected on ultrasound undergoing invasive testing as part of routine care recruited to study (REC 18/WA/0221).

2a. Germline DNA (gDNA) extracted from:
- mother
- father
- fetus (invasive)

2b. Cell-free DNA (cfDNA) containing cell-free fetal DNA (cffDNA) extracted from maternal plasma

3. Phenotype and HPO curation

4. Optimised targeted enrichment and sequencing of c.2000 genes

INGENIOUS gene panel:
-Fetal Anomalies (PanelApp)
-Literature curated genes describing prenatal phenotypes

5. Analysis to identify *de novo* variant(s) in invasive sample

6. Pipeline development to call in cfDNA:
- fetal fraction
- fetal sex
- paternity
- *de novo* variation

De novo variation (invasive)
v
De novo variation (non-invasive)

7. Pipeline optimisation and validation

Results

Cohort

- Over 80 families have been recruited to date.
- Includes cases with confirmed clinical single gene disorder (dominant and recessive modes of inheritance) and those with no molecular diagnosis

De novo variant detection in cfDNA - clinical controls

Sample ID	Gestation (weeks)	Fetal fraction	# clinical DNM	Gene	Condition	Clinical DNM detected in cfDNA?	REF/ALT (maternal+ fetal/fetal)
006	15	13%	1	*RAF1*	Noonan syndrome 5	Y	310/25
027	28	23%	1	*FGFR3*	Achondroplasia	Y	427/56
031	14	16%	1	*SOS1*	Noonan syndrome 4	Y	675/38
035	21	13%	1	*COL1A1*	Osteogenesis imperfecta	Y	627/40

- 100% clinical sensitivity has been obtained with our cases to date

De novo variant detection in cfDNA – false positive and false negative rate

Sample	# DNM in invasive	# cfDNA DNM Pre-filtering	# cfDNA DNM Post-filtering	Invasive DNM detected in cfDNA?
006	1	50	4	1
007	0	116	7	n/a
010	0	18	1	n/a
011	0	35	0	n/a
012	0	20	2	n/a
014	0	29	3	n/a
015	2	91	5	2
016	1	173	4	0
018	0	99	1	n/a
026	1	36	5	1
027	1	9	1	1
031	3	16	3	3
035	2	24	2	2
Total	13	716	38	12

- An average of 0.9 *de novo* variants were identified per invasive sample (range 0-3).
- Pre-filtering, an average of 55.1 putative *de novo* variants were identified per non-invasive (cfDNA) sample (range 9-173).
- A refined post-filtering strategy resulted in an average of 2.9 putative *de novo* variants being identified per non-invasive sample (range 0-5).
- 12/13 of all *de novo* variants were identified in cfDNA (true positive 92%)
- 1/13 *de novo* variants were not identified in cfDNA (false negative 8%)

Conclusion

- The INGENIOUS gene panel can be used to detect causative *de novo* variants in cfDNA. This is the most extensive non-invasive panel presented to date.
- Further work is ongoing to assess additional samples, refine filtering strategy and to extend analysis to recessive conditions.

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