Customizable non-invasive prenatal testing for single gene disorders using cell free DNA

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Introduction

The ability to predict the fetal genotype of an at-risk pregnancy utilizing cell-free DNA (cfDNA) has been a request of patients and clinicians since the introduction of cfDNA screening for fetal aneuploidy in 2011. Families at-risk would benefit from being able to predict the fetal genotype without an invasive procedure or associated risks to the pregnancy. However, the challenge is significant, as the genomic region of interest is reduced from a whole chromosome with aneuploidy testing to a single molecular variant. We have developed a novel platform for non-invasive prenatal testing of single gene disorders by cfDNA from a maternal blood sample. This platform has been validated and shown to accurately predict the fetal outcome (affected vs. unaffected) in a series of pregnant women who were carrying at-risk pregnancies.

Methods

Samples: Blood is collected from pregnant patients at ≥ 10 weeks gestational age in a Streck BCT® and transported overnight to the Progenity lab. The tubes are centrifuged to separate the plasma. Cell-free DNA is extracted from maternal plasma using an internally developed bead-based method.

Fetal fraction determination: A portion of the cfDNA is tested in triplicate using a NGS method for determination of the total fetal DNA contribution.

Fetal genotyping: The BIORAD QX200TM Droplet Digital PCR system is used to perform a variant-specific probe-based assay. Measurements of the relative abundance of reference and alternate alleles are produced, resulting in the cfDNA allele ratio.

Data analysis: The total fetal DNA contribution, the cfDNA allele ratio, and other run-based data are used to calculate the fetal status (affected vs. unaffected) and the associated probability.

Results

DESIGN OF PLATFORM

Model system: A benign variant model system was developed and used to validate our platform. A common variant identified as heterozygous was selected for each mother. The alleles for each loci were arbitrarily assigned as the disease causing variant or the wild-type variant.

Variant targeting: Target variants were made up of single nucleotide substitutions (SNPs), insertions (INS), and deletions (DEL). Insertions and deletions ranged from 2bp to 5bp in length. A unique variant was targeted for each sample.

Inheritance modes: The test includes a total of 57 women (gestational age 10-20 weeks), including 40 women who were heterozygous at the target variant, mimicking autosomal recessive and X-linked inheritance, and 17 women who were homozygous at the target variant, mimicking autosomal dominant inheritance or de novo mutations.

PLATFORM VALIDATION RESULTS

Concordance: In this blinded test, the fetal outcome data show 100% concordance (53/53) to neonate genotypes obtained via Sanger sequencing (Table 1).

Reportable rate: 53 of 57 samples passed all QC metrics and were definitively resulted. Four samples were resulted as inconclusive due to low fetal fraction or QC failure (Table 1).

TABLE 1. PLATFORM VALIDATION TESTING OF CONCORDANCE WITH KNOWN OUTCOME

Result	Sample Count	% of Total
Correct	53	92.98%
22 Affected, 31 Unaffected		
Incorrect	0	0.00%
Inconclusive	4	7.01%
Low FF (3.48%), Low FF (2.75%), Low FF (0.97%), QC Fail (Insufficient DNA)		

PATHOGENIC ASSAY TESTING

Here we tested pregnant mothers that were either known carriers or affected with autosomal recessive disorders. Custom assays were developed for each of the listed variants (Table 2), and plasma testing was conducted as detailed in the methods above. The assay reports fetal status as "affected" (i.e. homozygous for the maternal variant) or "unaffected" (i.e. heterozygous for the maternal variant or homozygous for the wild-type variant). When available, biological samples were collected from the resulting neonate and subjected to genotyping via Sanger sequencing to determine neonate concordance and confirm the custom assay results. In all cases, our results are concordant with the neonate outcome.

TABLE 2. CUSTOM ASSAY TESTING OF PREGNANT MOTHERS WITH KNOWN PATHOGENIC VARIANTS

Sample ID	Variant	Maternal Genotype	Gestation Age (wks)	Fetal Fraction	Fetal Status	Confidence	Neonate Concordance
023	*BLM* c.2207_2212delinsTAGATTC	Heterozygous	25	20.17%	Unaffected	99.90%	Yes
021	*CLRN1* c.144T>G	Heterozygous	25	11.91%	Unaffected	99.90%	Yes
012	*DHCR7* c.964-1G>C	Heterozygous	16.5	15.40%	Unaffected	99.90%	Yes
088	*DHCR7* c.964-1G>C	Heterozygous	18	11.80%	Unaffected	99.90%	Not available
046	*GJB2* c.167delT	Heterozygous	10.5	13.24%	Unaffected	99.90%	Yes
040	*GJB2* c.167delT	Heterozygous	20	8.71%	Unaffected	99.90%	Yes
075	*GJB2* c.167delT	Heterozygous	22	9.36%	Unaffected	99.90%	Not available
049	*GJB2* c.167delT	Homozygous	24.5	4.87%	Unaffected	99.90%	Yes
078	*GJB2* c.167delT	Heterozygous	25.5	13.06%	Unaffected	99.90%	Not available
004	*GJB2* c.35delG	Heterozygous	18	17.19%	Unaffected	99.90%	Yes
235	*HBB* c.20A>T	Heterozygous	15	6.41%	Unaffected	99.90%	Yes

Discussion

We have developed a platform for the non-invasive prenatal detection of monogenic diseases with >99.9% sensitivity and specificity and a 92.9% reportable rate. The platform maintains the high sensitivity and specificity seen with the model system (common variant targeting) when interrogating pathogenic variants associated with common monogenic disorders.

This test provides families with fast and accurate information about the status of a single gene disorder during pregnancy. This initial screening result allows them to make well-informed decisions about prenatal diagnostic testing and pregnancy and neonatal management.