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Genome-wide cfDNA testing: Clinical laboratory experience screening for sex chromosome abnormalities

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I. Introduction

Genome-wide cell-free DNA (cfDNA) testing in pregnancy was introduced for clinical use in August 2015. During the first year following its release, 16,670 samples were submitted for analysis. We describe the clinical laboratory experience of this screening test for the detection of sex chromosome abnormalities.

II. Methods

Maternal blood samples submitted to Sequenom Laboratories® for MaterniT® GENOME testing were subjected to DNA extraction, library preparation, and whole-genome massively parallel sequencing as previously described by Jensen et al[1]. Sequencing data were analyzed using a novel algorithm to detect aneuploidies and other subchromosomal events as described by Lefkowitz et al[2]. For all positive results, outcome data (e.g. cytogenetic/molecular results and/or birth outcomes) were elicited by phone or email from the ordering provider.

III. Results

Analysis of 16,670 samples yielded 158 results that were reported as positive for a sex chromosome abnormality, generating a positivity rate of 0.95%. Additionally, six results were reported as "uninformative" for SCA, but included a comment from the laboratory director regarding the unusual SCA data. In descending order, the most common SCAs reported were monosomy X (n=106), XXY (n=17), XXX (n=15), and XYY (n=8). There were four samples reported as positive for double aneuploidies involving the X chromosome, and another eight samples reported as positive for subchromosomal events involving the X chromosome. (Figure 1) Diagnostic follow-up information was available for 34.1% (56/164) of the positive/uninformative samples. Of those 56 samples, 41 diagnostic results were consistent with the cfDNA findings. Diagnostic results were defined as karyotype or microarray results from CVS and/or amniocentesis, POC specimens, or peripheral blood from the newborn or mother. When both CVS and amniocentesis results were available (n=1), CVS was considered ground truth for determining the accuracy of the cfDNA results due to the common biological origin of the DNA used during analysis of these tests. Thirty-four of the 41 results established an SCA diagnosis in the fetus, five results established an SCA diagnosis in the mother, and two results established an SCA diagnosis in both the mother and the fetus. (Figure 2) The five maternal diagnoses consisted of mosaic Turner syndrome (n=3), as well as subchromosomal events involving the X chromosome (n=2). The presence of a maternal SCA precluded prediction of the sex chromosome status of the fetus. Of the 15 discordant results, 11 were for monosomy X, two involved double aneuploidies in which the second aneuploidy (one trisomy 21 and one trisomy 18) was concordant by prenatal diagnosis, one involved a subchromosomal event, and one uninformative was a false negative for XXY. It is important to note that placental mosaicism is a well-documented cause of discrepant cfDNA results, and may exist even in the presence of normal fetal studies. The relative positive predictive value (PPV) of the test is 73.2% (41 confirmed diagnoses divided by 56 cases with diagnostic follow-up information). Excluding maternal events, the relative PPV is 64.3% (36/56). Of the 108 cases without diagnostic confirmation, 74.1% (80/108) had clinical findings (e.g. ultrasound findings and/or abnormal serum biochemical screening) to support the positive cfDNA result. Abnormal serum biochemical screening is a common feature of chromosomally abnormal placentas.[3-6] (Table 1) There were three false negative results reported to the laboratory from ordering providers. (Table 2) NOTE: One case was not readily classifiable. The fetus had multiple congenital anomalies. The pregnancy resulted from IVF after a three-embryo transfer, and the patient was taking Lovenox. Results were positive for trisomy 3 (predicted to be mosaic) and uninformative for SCA. The fetal karyotype was normal.

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Figure 1: Overview of MaterniT® GENOME results positive for a sex chromosome abnormality from August 31, 2015 to August 31, 2016

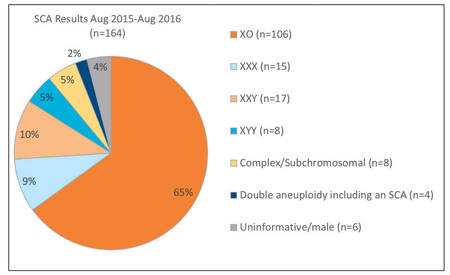

Figure 2: Breakdown of cases with diagnostic information available

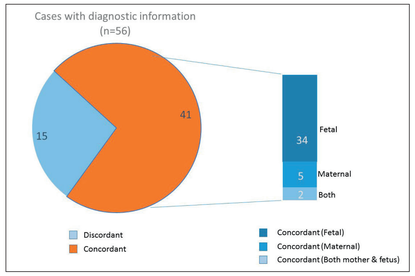

Table 1: Clinical outcomes of sex chromosome abnormalities identified by MaterniT® GENOME from August 2015 through August 2016

Sex Chromosome Abnormality Reported	Total Reported Positive	Concordant	Likely Concordant	Unknown	Discordant	PPV lower-upper estimate (%)	PPV from only cases with confirmed outcomes
XO	106	19 (16 fetal + 3 maternal)	62	14	11	76.4 – 89.6%	19/30 = 63.3%
XXX	15	5	5	5	0	66.7 – 100%	5/5 = 100%
XXY	17	6	4	7	0	58.8-100%	6/6 = 100%
XYY	8	3	3	2	0	75-100%	3/3 = 100%
Complex / Subchromosomal*	8	5 (2 maternal, 1 fetal, 2 confirmed in mother and fetus)	2	0	1	87.5%	5/6 = 83.3%
Double aneuploidy**	4	0	2	0	2	50%	0/2 = 0%
Uninformative for SCA (male fetus)***	6	3	2	0	1	83.3%	3/4 = 75%
Total	164	41	80	28	15	73.8 – 90.9%	41/56 = 73.2%

***= Complex/Subchromosomal:** One (or more) deletion/duplication ≥7Mb involving the X chromosome was reported.

****= Double aneuploidy:** T21/Monosomy X (n=1); T18/Monosomy X (n=1); T16/Triple X (n=1); T21/Triple X (n=1).

*****= Uninformative for SCA (male fetus):** Lab director comments included: Suspected maternal XO mosaicism (n=4); Extreme overrepresentation of X (n=1); No additional lab director commment (n=1).

Concordant: "True positive" – The positive cfDNA results were confirmed in the fetus or mother by karyotype and/or microarray analysis on samples from CVS, amniocentesis, POC, or peripheral blood.

Likely Concordant: "Suspected" – Cases in which diagnostic testing was declined, but clinical findings (e.g. ultrasound abnormalities, serum biochemical screening, maternal phenotype) support the abnormal cfDNA result.

Unknown: No additional information was available from the ordering provider.

Discordant: Positive cfDNA results with a normal fetal karyotype or microarray. Diagnostic studies were performed by amniocentesis (n=9), postnatal peripheral blood (n=2), POC (n=2), CVS (n=1), and unspecified prenatal diagnostic test (n=1).

PPV lower-upper estimate: Lower estimate is calculated by combining 'concordant' and 'likely concordant', divided by the total number of positives (presuming that all patients with no additional information are confirmed to be false positives). Upper estimate is calculated by combining 'concordant', 'likely concordant', and 'unknown', divided by the total number of positives (presuming all patients with no additional information are confirmed to be true positives).

PPV from only cases with confirmed outcomes: 'Concordant' divided by the sum of 'concordant' and 'discordant'.

Table 2: Description of false negative results

cfDNA Result	Clinical information	Follow-up information provided by ordering clinician
Negative, male	- Prior SNP-based NIPT negative - Anomalous fetus (NOS)	- Amnio karyotype: 48, XXY, +18 - Double aneuploidy
Negative, female	- Spontaneous miscarriage at 19 weeks gestation - Postnatal urogenital anomalies (NOS)	- POC testing: monosomy X, trisomy 16, and trisomy 22/tetraploidy 22 - Multiple aneuploidies - Paraffin tissue, believed to be villi
Positive for Trisomy 13, male - Possibly mosaic	- AMA - Abnormal serum biochemical screening - No reported ultrasound anomalies	- Amnio karyotype: mosaic Klinefelter syndrome (3 of 15 colonies)

IV. Conclusion

Genome-wide cfDNA testing allows for the identification of sex chromosome aneuploidies, as well as subchromosomal events involving the sex chromosomes. Though the uptake of diagnostic testing in these cases is limited, knowledge of a potential chromosome abnormality in these pregnancies may help families arrange for appropriate diagnostic testing at birth. Early diagnosis of sex chromosome abnormalities may improve long-term outcomes for these children.[7-14]

V. References

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