A review of cfDNA-detected sex chromosome anomalies with microarray and/or chromosome analysis follow-up

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1. Introduction

Non-invasive prenatal screening is a technology utilized by clinicians to provide screening results to patients for common trisomies and sex chromosome aneuploidies (SCAs) by analyzing cell-free fetal DNA (cfDNA). As this testing is utilized more frequently, it is important for clinicians to understand the variety of complex results that can arise from follow-up diagnostic testing of SCA screen-positive pregnancies and the capabilities of laboratory assays to detect these findings. This study examines the complexity of diagnostic follow-up results obtained via prenatal chromosome and microarray analysis.

2. Methods

Specimens and ascertainment

Cases with an indication of SCA screen-positive cfDNA were identified from a data search of prenatal specimens ordered for standard chromosome analysis, fluorescence in situ hybridization (FISH) or microarray studies. Diagnostic testing was ordered at the discretion of the referring provider. These samples were received from Labcorp clients throughout the United States and internationally from 2013 to June 2022. Chromosome analysis and FISH studies were performed using standard analyses. For microarray studies, amniotic fluid was set up as a direct (uncultured) specimen if the gestational age was 15 weeks or greater and at least 15 mL of fluid was available. If the gestational age was 17 weeks or greater, only 10 mL of fluid was needed. For chorionic villi samples (CVS), direct specimens were analyzed if more than 10 mg of CVS material was available. For all direct specimens, back-up cultures were established and utilized in case of direct analysis failure. Cultures could be established from as little as 5 mL of amniotic fluid or 2 mg of CVS material. This study focused on fetal diagnostic results as follow-up to cfDNA screening and did not extend to maternal cytogenetic analyses that may have been ordered by referring providers as follow-up to cfDNA screening.

All cases were placed into one of five SCA screen-positive groups as designated by cfDNA results: cfDNA positive for: 1) monosomy X; 2) XXX; 3) XXY; 4) XYY; or 5) other sex chromosome anomaly not previously mentioned.

Array methodology

All microarray studies were performed utilizing the Affymetrix® Cytoscan® HD array [Affymetrix® and CytoScan® are Registered Trademarks of Thermo Fisher Scientific]. This array contains approximately 2.695 million markers across the entire human genome. There are approximately 743,000 SNPs and 1,953,000 structural non-polymorphic probes (NPCNs). On average, there is approximately 0.88 kb between each marker. DNA was extracted utilizing standard methods. 250 ng of the total genomic DNA extracted was digested with NspI, then ligated to NspI adaptors, and amplified using Titanium® Taq with a GeneAmp® PCR System 9700. PCR products were purified using AMPure beads and quantified using NanoDrop 8000. Purified DNA was fragmented, biotin labeled and hybridized to the Affymetrix Cytoscan® HD GeneChip. Data was analyzed using Chromosome Analysis Suite. The analysis is based on the GRCh37/hg19 assembly.

The SNP array analysis is utilized to detect both copy number changes as well as copy neutral changes. This allows the detection of not only deletion and duplication, but also potential uniparental disomy and identity by descent. The presence of SNPs in the microarray also allows detection both of triploidy and complete moles with total homozygosity.

3. Results

Types of sex chromosome anomalies identified

A total of 520 chorionic villi or amniotic fluid samples from patients who screened positive for SCAs (monosomy X, XXX, XXY, XYY and "other SCAs") were included. The majority of the abnormal diagnostic results yielded full aneuploidy findings consistent with their referral indication (63.79%); however, some cases, especially in the monosomy X positive group, had a variety of complex findings that required both chromosome analysis and microarray to clearly define. Mosaic and structural abnormalities involving not only the X chromosome, but also the Y chromosome, were seen in this group. These findings included mosaic isodicentric chromosomes involving X or Y, ring X, large X deletions, X/Y translocations, and monosomy X mosaicism with an additional normal or abnormal sex chromosome cell line. See **Figure 1** for a visual representation of the types of anomalies detected for each cfDNA screen-positive group. Please note that none of the cases in the "other SCA" positive category were confirmed on diagnostic testing and were not included in **Figure 1**.

Discrepancies in diagnostic results due to mosaicism

In 18 cases, chromosome and microarray analyses were discrepant with only one of these diagnostic tests identifying a mosaic cell line. Seventeen of these cases were positive for monosomy X on cfDNA, and one case was positive for XXX (case 18 in **Table 1**). **Table 1** displays the results of the FISH analysis (if performed), chromosome analysis and microarray analysis for these 18 cases. Three of these cases were chorionic villi samples (CVS), where the remaining 15 were amniotic fluid samples. One of the three mosaic CVS cases had a normal follow-up amniotic fluid analysis; a data search did not reveal follow-up for the other two CVS cases. One case (case 13), identified to have additional material on the Y chromosome in one cell line, was followed up by cord blood studies after delivery and yielded a finding of an unbalanced translocation [t(Y;2)(q12;q15)].

Incidental microarray findings

Thirty-six of the 520 cases (6.92%) had an abnormal microarray result that did not involve a SCA or a structurally abnormal sex chromosome. These cases varied in their initial cfDNA screen-positive category. The incidental microarray results included regions of homozygosity (ROH) that indicated uniparental disomy (UPD) of the X chromosome or another chromosome, identity by descent (long ROH of >8 MB on two or more chromosomes) or similar parental ancestry (1-8 MB ROH on multiple chromosomes with an elevated ROH total). Also found were variants of uncertain significance, deletions of autosomal recessive genes, copy number variants (CNVs) associated with known phenotypes, complex unbalanced rearrangement of an autosome, supernumerary markers and other mosaic aneuplodies. See **Figure 2** for more information.

4. Conclusions

cfDNA screening has the potential to detect full sex chromosome aneuploidy. Additionally, this study emphasizes the importance of counseling patients to the possibility that diagnostic testing could also identify a mosaic or complex sex chromosome anomaly that might not be inferred by the cfDNA screen result. Follow-up diagnostic studies are needed for all positive screen results to determine if an SCA is present and if the finding is a full SCA or a more complex finding. This is particularly evident for those pregnancies that screen positive for monosomy X. This study demonstrates how both chromosome and microarray analyses should be utilized by clinicians to clearly define and provide accurate results to a patient and to accurately assess for mosaic results. Furthermore, microarray analysis can reveal additional complexity of sex chromosome aberrations as well as detect incidental findings that may have otherwise not been identified.

Tables + Figures

Figure 1. Types of SCA identified via diagnostic testing

Of the cases in which an SCA was confirmed via diagnostic testing, these cases were further examined to determine the type of SCA identified and whether this was consistent with the SCA risk identified by cfDNA analysis. The monosomy X screen-positive cfDNA category yielded a variety of different types of SCA diagnoses as well as complex findings that required both a chromosome analysis and microarray to clearly define.

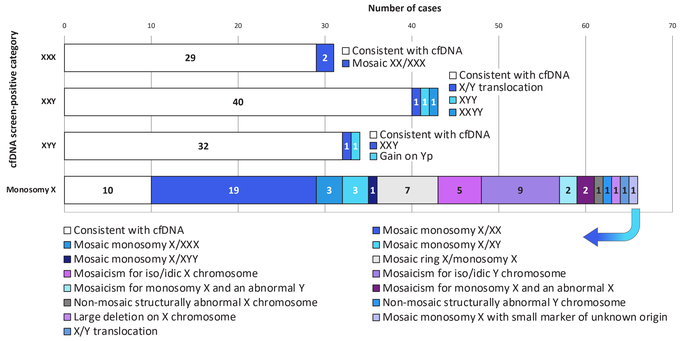
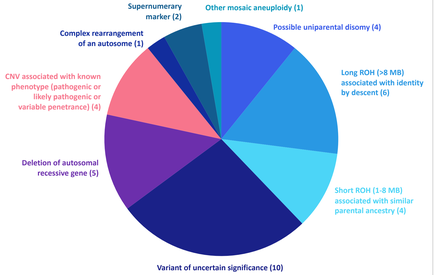


Figure 2. Incidental findings on microarray



Thirty-six of the 520 cases (6.92%) had a microarray finding that was not consistent with a SCA, but provided a different abnormal finding. Of note, one of these cases had two non-SCA findings, possible UPD and a CNV associated with a known phenotype.

Table 1. Discrepancies in diagnostic results due to mosaicism

Case	Microarray sample type	FISH analysis	Chromosome analysis result	Microarray analysis result	Additional testing/follow-up
Case 1	Direct chorionic villi	Monosomy X in 50 cells	46,XX	Mosaic monosomy X (35% of cells with monosomy X)	Amniotic fluid FISH, chromosome analysis and microarray yielded a 46,XX/normal female result
Case 2	Direct chorionic villi	None	46,XX	Mosaic monosomy X (72% of cells with monosomy X)	No amniotic fluid follow-up found
Case 3	Direct chorionic villi	Monosomy X	46,XX	Mosaic monosomy X (14% of cells with monosomy X)	No amniotic fluid follow-up found
Case 4	Direct amniotic fluid	Normal male	45,X[4]/46,XY[14]	Normal male	
Case 5	Direct amniotic fluid	Uninformative	45,X	Mosaic for monosomy X and a ring X (ring present in 62% of cells)	
Case 6	Direct amniotic fluid	Uninformative	45,X[2]/46,XY[3]	Mosaic for monosomy X (25% of cells) and isodicentric Y chromosome (75% of cells)	SRY FISH confirmed the isodicentric chromosome Y
Case 7	Amniotic fluid culture	None	46,XX in 22 colonies, one 45,X colony was observed in one primary culture	Normal female	
Case 8	Amniotic fluid culture	Normal female	47,XX,+i(X)(q10)[4]/46,XX[26]	Normal female	
Case 9	Amniotic fluid culture	Normal female	45,X[2]/46,XX[13]	Normal female	
Case 10	Amniotic fluid culture	Uninformative	45,X[5]/46,XX[10]	Normal female	
Case 11	Amniotic fluid culture	Uninformative	45,X[3]/46,XX[20]	Normal female	
Case 12	Amniotic fluid culture	None	45,X	Mosaic monosomy X (70% of cells)/idic Xq (30% of cells)	
Case 13	Amniotic fluid culture	Targeted FISH studies utilizing Y chromosome probes showed the marker was derived from the Y chromosome: ish add(Y)(q12)(SRY+,DYZ3+,DYZ1+)	46,X,+mar[8]/45,X[4] Chromosomes were revised to: 46,X,add(Y)(q12)[8]/45,X[4]. ish add(Y)(q12)(SRY+,DYZ3+,DYZ1+)	Monosomy X	Testing on cord blood at delivery. Microarray identified a non-mosaic unbalanced translocation derivative Y with partial trisomy of the 2p15-pter segment and partial monosomy of the distal long arm Y involving the PAR2 region
Case 14	Amniotic fluid culture	XXX pattern	45,X[10]/47,XXX[5]	Normal female (Comment on array that a 50:50 mix of 45,X/47,XXX would result in a normal female pattern on the array)	
Case 15	Amniotic fluid culture	Normal female	46,X,+mar[3]/46,XX[17]	Normal female (no evidence of loss of X or a marker)	
Case 16	Amniotic fluid culture	Normal female	46,XX	Mosaic monosomy X (15-30% of cells with monosomy X)	
Case 17	Amniotic fluid culture	Normal male	45,X[2]/46,XY[15]	Normal male	
Case 18	Amniotic fluid culture	XXX pattern	47,XXX	Mosaic gain of X chromosome in 67% of cells	

Table 1. Cases in which diagnostic results were discrepant between chromosome analysis and microarray. FISH results are displayed for those cases for which testing was ordered. FISH refers to aneuploidy FISH unless otherwise indicated. The cases are grouped and color coded based on sample type utilized for the microarray analysis. It is noted whether the microarray analysis was performed on a direct or cultured sample as well as if the sample was a chorionic villi sample (CVS) or amniotic fluid (AF) sample.

National Society of Genetic Counselors Annual Conference, November 16-20, 2022, Nashville, TN

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