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The Impact Of Microarray Studies On Prenatal Diagnosis: Analysis Of 50,000 Prenatal Samples And 15,000 POCs

Stuart Schwartz, Rachel Burnside, Inder Gadi, Karen Phillips, Mary K. Rudd, Justin Schleede, James Tepperberg, Peter Papenhausen

Integrated Genetics, Laboratory Corporation of America® Holdings

Poster presented at the *10th Prenatal Diagnosis: Ultrasound/Fetal ECHO, Genetics & MFM/OB Conference*; 2018 June 6-9; Philadelphia, PA.

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I. INTRODUCTION

Over the past ten years cytogenetic diagnosis has evolved with the use of arrays. Microarray analysis is now well established for the genetic evaluation of pediatric patients; however, its utilization for prenatal diagnosis and POC specimens is still somewhat limited and not always universally accepted. This study addresses 50,000 prenatal patients and 15,000 POCs that were studied by microarray analysis (Affymetrix® Cytoscan® HD) to detect aberrations in prenatal patients with AMA and those with ultrasound abnormalities not detected by chromosome analysis, as well as to determine its overall utilization in POC analysis. The present study reviews a large laboratory's experience with utilization and efficacy of microarray analysis for prenatal and POC diagnosis to illustrate its efficacy and importance as a first-tier test.

OBJECTIVES:

The overall objectives of this work were to determine if SNP microarray analysis from prenatal and POC specimens could:

- Determine the utilization and efficacy of this technology in patients with ultrasound abnormalities
- Determine the utilization and efficacy of this technology in patients with advanced maternal age/anxiety
- To enumerate the advantages and pitfalls of this technology
- To provide an overview of a large laboratory's experience with microarray analysis for prenatal diagnosis and POC and delineate it's utilization as a first-tier test.

II. MATERIALS AND METHODS

SPECIMENS AND ASCERTAINMENT: This study addresses 50,000 prenatal and 15,000 POC specimens that were studied by microarray analysis to detect aberrations. Amniotic fluid or Chorionic samples were obtained for standard cytogenetic analysis in some but not all cases. Cytogenetic and FISH studies were done at the discretion of the referring provider. Cytogenetic and FISH studies were done using standard analyses, but were not done for all specimens. For microarray studies, DNA could be extracted from as little as 1 cc, although more is preferred. These were broadly grouped into the type of sample and the ascertainment of those patients.

ARRAY METHODOLOGY: All studies were done utilizing the Affymetrix® Cytoscan® HD array [Affymetrix® and CytoScan® are Registered Trademarks of Affymetrix, Inc.]. 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 the average there is approximately 0.88 kb between each marker. DNA was extracted utilizing standard methods and 250ng of total genomic DNA extracted was digested with NspI and 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, and 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. Criteria for reporting copy number changes were deletions greater than 1.0Mb and duplications greater than 2.0Mb. Deletions or duplications as small as 25kb were also reported in custom genes of known cinical significance. Possible CN-LOH was reported when a telomeric homozygous block greater than 8 Mb was present and 15 Mb interstitial block for imprinted chromosomes (10 and 20 Mb for non-imprinted chromosomes).

III. RESULTS

Overall the analyses were successful greater than 99.5% of the time in prenatal patients and > 95% in POCs. For prenatal patients, approximately 75% of the specimens were amniotic fluid (AF) and 68% of the specimens were direct (not cultured) analyses. Referrals for an abnormal ultrasound finding (U/S) were the most frequent indication (~58%) followed by AMA (~31%). Although it did not account for referrals in the initiation of our studies, cfDNA is now the third most common referral. Microarray analysis detected cytogenetic abnormalities (when chromosomes were normal) for both AMA (1.7%) and abnormal U/S (~4.3%). This was even greater when the U/S revealed major ultrasound abnormality (~9.2%) [Table 1]. Over 83% of the aberrations detected were smaller than 5 Mb. Variants of unknown significance (VOUS) were detected in only 1~2% of both prenatal and POC patients and were familial 90~95% of the time. For POCs, the vast majority are from direct villi specimens, although ~20% are from paraffin specimens. Among the normal POC specimens, there was almost a 50:50 male:female split demonstrating the effectiveness of direct specimens yielding fetal results. Additionally, the frequency of structural aberrations was much greater than expected (Table 2).

IV. TABLES AND FIGURES

Table 1: Frequency of array findings in selected ultrasound abnormalities

	PATHOGENIC	IBD	UPD	TOTAL
Major	9.2%	2.9%	0.8%	12.9%
Major - Heart	8.1%	2.5%	0.3%	10.9%
Multiple Anomalies	4.3%	4.8%	0.1%	9.2%
Multiple Anomalies – Heart	8.2%	3.8%	0.8%	12.8%
Nuchal Translucency	2.5%	3.0%	0.3%	5.8%
Diaphragmatic Hernia	6.2%	2.1%	0.5%	8.8%
Holoprosencephaly	6.9%	2.5%	2.9%	12.3%

Internal Data (January, 2018)

Table 2: Relative frequency of different types of CNVs in POCs

TYPE OF ABERRATION	ALL POCs	POCs > 25 WEEKS
STRUCTURAL < 5 Mb	9.4%	42.6%
STRUCTURAL >5 Mb	5.5%	4.9%
ANEUPLOID	60.3%	48.3%
TRIPLOID	12.2%	1.6%
MONOSOMY X	12.6%	2.5%

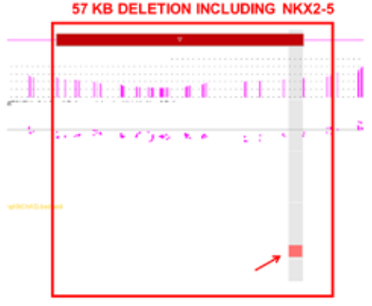

Figure 1: 25 year old female with a 24.3 week gestation referred with possible hypoplastic left heart syndrome. Cytogenetics -46,XX; Array revealed a 57 kb de novo interstitial intragenic deletion of the NKX2-5 gene

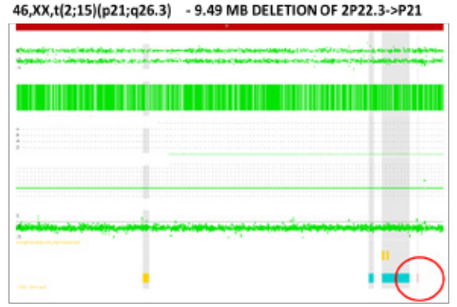

Figure 2: 34 year old female referred for abnormal ultrasound (holoprocencephaly). Cytogenetics - 46,XX,t(2;15)(p21;q26.3); Array revealed a 9.49 Mb deletion of 2p22.3->p21 including a SIX2 gene deletion

V. CONCLUSIONS

These findings have provided compelling results for the utilization of a SNP microarray with both prenatal and POC samples. Using this technology, abnormalities in chromosomally normal patients or patients where cytogenetic studies failed, especially in POCs were achieved. We were also able to clarify additional chromosomal anomalies and provide better information. In addition, SNP microarray technology provided for the detection of homozygosity which has been shown to detect uniparental disomy and apparent common descent.

The results from the analysis of 50,000 prenatal microarray patients and 15,000 POCs, has revealed several important findings:

1. It is clear that microarray analysis should be a first-tier test for both prenatal and POC specimens;
2. The success rate, especially for POCs is greater than by conventional methods and is more likely to involve fetal tissue;
3. The frequency of VOUS can be kept low (by using carefully considered criteria) and the vast majority are familial;
4. In prenatal patients, a high frequency of aberrations were in patients ascertained with multiple anomalies and a heart defect (~8%), with a surprising >90% not involving a 22q deletion;
5. Although the majority of abnormal POC specimens involved a whole chromosome, there was an increase in unbalanced structural rearrangements and small pathogenic CNVs not detectable by standard studies.

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