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SNP microarray analysis of over 20,000 products of conceptions (POC): implications, importance and suggestions for standard of care

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1. Introduction

Microarray analysis has been an important technology in the cytogenetics laboratory over the past decade. This technology has the capability to detect small copy number gains and losses not seen with standard chromosome analysis, as well as, alterations involving homozygosity associated with identity by descent and uniparental disomy. Although microarray testing is now usually recommended as the first genetic test for pediatric patients and has been utilized for prenatal samples over the past 8 years, it is still not universally utilized for the study of POC specimens. In this study, we report on one of the largest number of POC specimens utilizing a SNP microarray (Cytoscan® HD – Applied Biosystems™) analysis of over 20,000 tissue specimens, and Oncoscan® (Applied Biosystems™) analysis of over 4,000 FFPE specimens. This study not only indicates the efficacy and usefulness of this technology, but also highlights several novel and unusual findings, as noted below.

2. Methods

Array Methodology – Cytoscan®: All studies on fresh tissue and some FFPEs (Formalin-fixed paraffin embedded tissue) were done utilizing the Affymetrix® Cytoscan® HD array [Affymetrix® and CytoScan® are Registered Trademarks of ThermoFisher 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 the average there is approximately 0.88 kb between each marker. DNA was extracted utilizing standard methods and 250 ng of total genomic DNA was digested with NspI, ligated to 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 the Chromosome Analysis Suite. The analysis is based on the wGRCh37/hg19 assembly.

Array Methodology – Oncoscan®: The majority of the studies on FFPEs were done utilizing the Affymetrix® Oncoscan® array [Affymetrix® and Oncoscan® are Registered Trademarks of ThermoFisher Scientific]. This array contains approximately 220,000 SNP probes with a median spacing of 5.0 kb, within the majority of genes. Total genomic DNA extracted was incubated with the annealed MIP (Molecular Inversion Probe). Amplified MIP product was digested with HAEIII and hybridized to the OncoScan® FFPE GeneChip. Data was analyzed using the Chromosome Analysis Suite. The analysis is based on the GRCh37/hg19 assembly.

3. Results

There were approximately 20,618 POCs (with fresh tissue) studied by Cytoscan® and 1,673 FFPE specimens studied by either Cytoscan® or Oncoscan®. When information was available these were grouped by gestation and collated into 1st Trimester (less than 13 weeks), 2nd trimester (13-22 weeks) or Stillborn (22 weeks or greater). For POCs ~16% did not have a gestational age available, 40% were in the 1st trimester, 27% in the second trimester and 16% stillborn. The FFPE specimens had a higher percent without a gestational age available (30%), 42% were in the 1st trimester, 16% in the second trimester and 11% stillborn.

As expected the frequency of abnormalities varied based on the gestation age (**Table IA** and **IB**). For POCs, 54.6% were abnormal if ascertained in the 1st trimester, 16.1% in the second trimester and 10.3% stillborn. For FFPE specimens there were similar findings with 53.1% abnormal in the 1st trimester, 16.7% in the second trimester and 13.8% of the stillborn.

In addition to the difference in frequency of chromosomal aberrations in the timing of pregnancy loss, there are stark differences between the types of abnormalities seen in the first trimester versus those detected in stillbirths. Table 2 illustrates the overall frequency of the different types of abnormalities detected. There are two stark differences: The frequency of autosomal trisomies (of the total abnormalities) in first trimester is 66.9%, where as in stillbirths is only 44.7%. In contrast, the frequency of pathogenic structural abnormalities (of the total abnormalities) in stillbirths is 34% (just slightly less than autosomal trisomies) while in first trimester only 5.7% (**Table 2**).

Autosomal Trisomy: An examination of the pattern of trisomies is intriguing in both 1st trimester pregnancy loss and in stillbirths. All of the autosomal chromosomes were detected in 1st trimester losses. The least common were for chromosomes 1, 17 and 19. The most common trisomy is chromosome 16, whereas the highest frequency also include chromosome 13, 15, 21 and 22. Both trisomies for chromosome 15 and 22 are more frequent than either trisomy 13 or trisomy 21. Approximately 7.2% of the autosomal trisomies in 1st trimester involve more than one chromosome. In contrast, only 1% of autosomal trisomies in stillbirths involve more than one chromosome. Seventeen different chromosomes have been detected in stillbirth, but 75% of the trisomies in stillbirth involve chromosome 13, 18 and 21, but only 8% in chromosomes 15, 16 and 22 the most common trisomies in 1st trimester losses.

Autosomal Monosomy: Autosomal monosomy makes up a small proportion of the abnormalities in 1st trimester loss (1.4%), but are still a significant cause. Monosomies involving chromosomes 1, 6, 7, 11, 21 and 22 were identified, however, monosomy 21 was involved in most of these comprising ~86% of these cases. There were no stillborn pregnancies in which an autosomal monosomy was detected.

Sex Chromosome Abnormality: The frequency of monosomy X (of the total abnormalities) is similar in both 1st trimester losses (12.3%) and stillbirths (12.9%), indicating these are important in the loss of pregnancies at any gestation. The low frequency of other sex chromosome aberrations indicate that they are not a factor in pregnancy loss.

Structural Abnormalities: Although the majority of abnormalities in pregnancy loss involve whole chromosome gains or losses, structural aberrations make up an important contribution to pregnancy loss. Pathogenic structural abnormalities (including microdeletion/duplication syndromes) comprise 5.7% of the abnormalities detected in 1st trimester abortions, but 34% of the abnormalities detected in stillborn pregnancies. Overall, the frequency of all patients with pathogenic CNV, having at least one CNV not detectable by standard chromosome analysis, is ~50%. Approximately 30% of these are neurodevelopmental microdeletion/duplications (e.g. 1q21.1, 16p11.2) and these have much broader implications for the family.

Utilization of Allele Difference and the B-allele: Although the smooth signal and log2ratio is utilized for determining copy number gain and loss, these are not always satisfactory to determine if there is an actual abnormality. In some cases, either the allele difference or B-allele can be utilized to make a diagnosis, clarify copy number change findings or assist in understanding the underlying mechanism leading to the abnormality.

Triploidy: Triploidy cannot be delineated using the log2ratio and smooth signal; however, analysis of allele patterns (demonstrating four instead of three tracks) can delineate this abnormality. Additionally, in triploid males, the parental origin can also be determined to rule out a possible paternal origin associated with a partial mole (**Figure 1**). Approximately 12.6% of the abnormalities in the 1st trimester are triploid. In contrast, only 4.3% of the abnormalities in stillborn pregnancies are triploid.

Tetraploidy: The majority of tetraploid cases cannot be detected by this technology as most of tetraploid cases result from a doubling of a diploid fetus and a diploid cannot be differentiated from a tetraploid. However, there were two cases in the first trimester that resulted from the fertilization by three sperms.

Complete Moles: Complete moles were detected in both 1st trimester pregnancy losses (0.7%) and in stillbirths (0.2%). The majority are female with two X chromosomes from a sperm that has doubled and without any maternal genetic component. This can be delineated by a normal dosage with complete homozygosity. A few cases develop from two sperms (with no maternal component) and demonstrate homozygosity in approximately half of the genome.

Mosaic Trisomy: Overall approximately 8.3% of the autosomal trisomies detected were mosaics, with the trisomy mosaicism ranging from anywhere from 10-80% of the sample. Many of these mosaics may not be detected by standard chromosome analysis because of possible overgrowth of normal tissue. However, these can be detected and delineated by the array. Additionally, some of these mosaic cases appear to have associated UPD (as delineated by the allele tracks) suggesting that the cases failed to complete UPD (**Figure 2**).

Maternal Cell Contamination: No matter how well POC samples are cleaned, maternal cell contamination (MCC) can be a problem in the analysis of these samples. Using standard cytogenetic analysis, MCC can result in overgrowth of maternal tissue and result in a normal 46,XX finding. The array analysis will utilize the alleles to illustrate the presence and estimate the extent of MCC, while still allowing the detection of abnormalities. Approximately 5.5% of the specimens demonstrated MCC, with the majority of these in the 1st trimester specimens.

Chimeric Specimens: Approximately 18 different specimens with chimerism were detected and found in the 1st and 2nd trimester and stillborn specimens. Most of these are a combination of a complete mole and biparental cell line (**Figure 3**). These chimeras that are of paternal origin have been recognized in cases of placental mesenchymal dysplasia (PMD) and molar pregnancies.

Variants of Uncertain Significance: One difficulty with microarray analysis is the possibility of getting unexplained results (or variants of uncertain significance). By applying strict POC reporting criteria, variants of uncertain significance (VUS) are only reported in 1~3 % of POC patients.

4. Conclusions

This study not only indicates the efficacy and usefulness of this technology but specifically highlights a number of unusual findings that have been elucidated. The findings in the current study have broad implications of the utilization and interpretation of POC microarray analysis.

- It is by far one of the most definitive studies to date conclusively showing a high yield in 1st trimester pregnancy loss and an increased yield of anomalies in stillbirths.
- Essentially all tissue samples could be studied by microarray analysis as long as enough DNA was available for analysis.
- It illustrates the importance of the detection of structural anomalies in all pregnancy loss, but especially in stillbirths.
- There is a higher frequency of neurodevelopmental susceptibility microdeletion/duplications detected than originally anticipated which makes counseling problematic but necessary.
- The array analysis also allowed detection of anomalies below the resolution of cytogenetic analysis, which includes approximately 50% of the structural anomalies detected.
- The frequency of VUS is 1~3% and most questions regarding these findings can be resolved with the appropriate parental studies.
- Microarray analysis allowed the detection of numerical anomalies that in standard cytogenetic analysis could have been overgrown by either normal cells or maternal cells.
- Close examination of the results reveal underlying mechanisms leading to the pregnancy loss including failed trisomy rescue; telomere capture; chimerism resulting in placental mesenchymal dysplasia as well as partial and complete moles.
- Overall all of these findings and conclusions continue to show that microarray analysis should be used for first tier testing.

Tables + Figures

Table 1A. Fresh tissue ascertainment and abnormal frequency

	1st Trimester	2nd Trimester	3rd Trimester
Number (%)	8282 (40.0%)	6908 (27.3%)	2064 (16.3%)
Abnormal (%)	54.6%	16.1%	10.3%

Table 1B. FFPE specimen ascertainment and abnormal frequency

	1st Trimester	2nd Trimester	3rd Trimester
Number (%)	708 (42.3%)	269 (16.1%)	189 (11.3%)
Abnormal (%)	53.1%	16.7%	13.8%

Table 2. Frequency and distributions of types of abnormalities

	1st Trimester	Stillbirth
Autosomal Trisomies	66.9%	44.7%
Autosomal Monosomies	1.4%	0.0%
Monosomy X	12.3%	12.9%
Other Sex Chromosome Abnormalities	0.3%	2.1%
Triploid	12.6%	4.3%
Tetraploid	0.04%	0.0%
Structural Aberrations	5.7%	34.0%
Molar Pregnancy	0.7%	0.2%

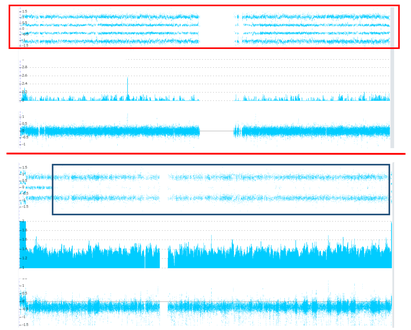


Figure 1A. Demonstrates the four track allele pattern (with chromosome copy number=2.0) seen on all autosomal chromosomes indicative of a triploid

Figure 1B. Demonstrates a long segment of homozygosity on the X chromosome (with chromosome copy number=1.33) indicative of the maternal origin of the extra haploid set of chromosomes

Figure 2. Mosaic trisomy 12 with ~17% gain. In addition, there is a 66.6 Mb region of homozygosity suggestive of UPD

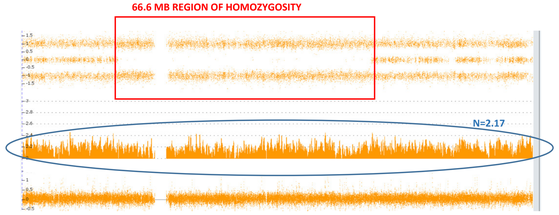
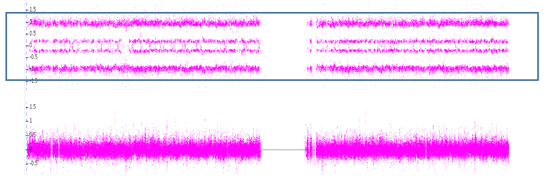


Figure 3. Demonstrates the four track allele pattern (with chromosome copy number=2.0) seen on all autosomal chromosomes, similar to that seen for a triploid. However, the spacing is different and microsatellite studies confirmed a chimera (between a homozygous mole and biparental line).



25th ISPD Virtual Annual Conference; 2021 June 6-8