# Products of conception analysis by chromosomal microarray: a single center experience.

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## INTRODUCTION

Chromosome aberrations detectable by conventional cytogenetics are a common cause of missed abortions and intrauterine fetal demise. However, chromosome analysis of products of conception (POC) may be unsuccessful or uninformative due to maternal cell contamination (MCC) and reduced viability of fetal cells in culture. Chromosomal microarray (CMA) may circumvent such issues, as it does not require culturing; in addition, MCC studies may be performed on extracted DNA prior to running the array. If those data indicate significant MCC, testing may be cancelled to avoid incurring the expense for a likely false negative result. A retrospective review of data from 484 cases submitted to Baylor Genetics for targeted chromosomal microarray analysis (CMA) following a stillbirth or a spontaneous loss of pregnancy was undertaken to assess clinical utility and determine best practices.

## METHODOLOGY

Samples submitted following pregnancy loss were triaged for amount and type of tissue as well as for test orders. The most common tissue types received were placental tissue including chorionic villi and cord; on rare occasions, skin was submitted. If CMA was the only ordered test on fresh tissue, a direct sample was sent for DNA extraction, and if available, a piece of tissue was frozen for additional extraction if needed. When chromosome analysis was also ordered, every effort was made to send a direct sample to extraction before initiating cultures. FFPE samples were also accepted for testing.

Prior to DNA extraction, the sample was incubated overnight at 37°C in Puregene Cell Lysis Buffer combined with 3 µl of Proteinase K (20mg/mL) and 1.5 µl RNaseA (100.0mg/mL). DNA extraction was performed using a modified Qiagen method as described previously (Breman et al 2012).

CMA was performed on a custom designed 180k array chip from Agilent containing 60,000 SNP probes for detection of AOH and triploidy in addition to >100,000 oligonucleotide probes targeting virtually all the known microdeletion or microduplication syndromes as well as the pericentromeric and subtelomeric regions with an average probe density of 10–20 kb/probe in the targeted regions. The average probe density over the entire genome (between disease regions) is 30 kb/probe.

Data was analyzed with proprietary in house software (Figure 1) until January, 2023 at which time, the use of NxClinical software was initiated. Reporting criteria have changed and continue to evolve; thus copy number variants have been reported for some later samples.

Receipt of a maternal blood sample allowed the degree of maternal cell contamination (MCC) to be evaluated by comparative analysis of maternal and fetal DNA using multiple unlinked polymorphic markers. CMA was not performed on samples showing ≥75% MCC. Figure 1B illustrates a diagnosis made in the face of ~50% MCC.

## RESULTS

484 samples submitted for targeted CMA

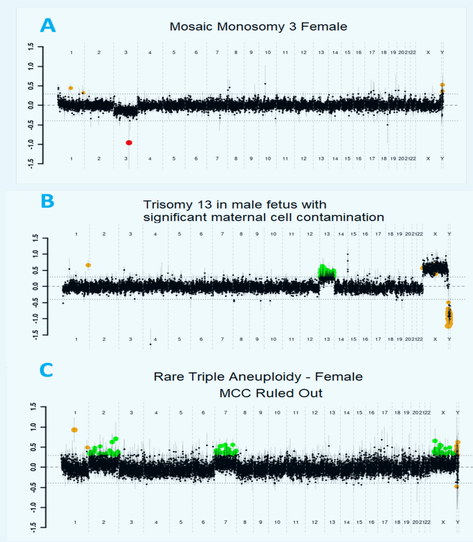
- 84 cancellations issued by the laboratory
  - 62 showed 75-100% MCC
  - 22 cases yielded insufficient quantity/quality of DNA after multiple extractions
- 400 CMA results issued
  - 152 normal male samples
  - 130 reported as normal female
    - 77 were negative for maternal cell contamination
    - 12 with <5% MCC
    - 7 with 6-10% MCC
    - 3 with 10-20% MCC
    - 5 with 20-50% MCC
    - 2 with 100% MCC (maternal sample submitted after a normal female report)
    - 24 cases had no maternal sample submitted
  - 118 reported with an abnormal or variant result
    - Table 1 shows breakdown by type of abnormality and gestational age
      - As expected, the majority of aneuploidies occur in the 1st and 2nd trimesters
      - Trisomy 21 is equally represented across all gestational ages
    - Table 2 shows specific aneuploidies and triploidies by gestational age
    - Table 3 shows clinically significant CNVs unlikely to be the etiology of the loss

## TABLE 1

| Trimester | | | | | |
|---|---|---|---|---|---|
| Gestational Age | 1st | 2nd | 3rd | Unknown | Total |
| Total # of Cases | 124 | 182 | 75 | 19 | 400 |
| # Aneuploid Cases | 49 | 24 | 8 | 4 | 85 |
| Triploidy | 7 | 1 | 0 | 0 | 8 |
| # Structural Abn. Cases | 1 | 2 | 0 | 0 | 3 |
| # CNVs | 3 | 11 | 8 | 0 | 21 |

**Table 1:** Breakdown of results by type of abnormality and gestational age. The gestational age was not provided in 19 cases. Data does not include samples that were unable to undergo CMA analysis but for which chromosome analysis was performed.

## FIGURE 1: CMA PLOTS




## TABLE 2

| Aneuploidy | Trimester | | |
|---|---|---|---|
| | 1st | 2nd | 3rd |
| Monosomy X | 14* | 4 | 0 |
| Trisomy 22 | 9 | 0 | 0 |
| Triploidy | 7 | 1 | 0 |
| Trisomy 15 | 5 | 0 | 0 |
| Trisomy 21 | 5 | 6** | 5 |
| Trisomy 13 | 4 | 3 | 0 |
| Trisomy 9 | 3** | 2 | 0 |
| Double Aneuploidy | 2 | 1 | 0 |
| Trisomy 8 | 1 | 0 | 0 |
| Trisomy 4 | 1 | 0 | 0 |
| Trisomy 14 | 1 | 0 | 0 |
| Trisomy 16 | 1 | 1 | 0 |
| Trisomy 18 | 1 | 4 | 2 |
| Trisomy 20 | 1 | 0 | 0 |
| Triple Aneuploidy | 1 | 0 | 0 |
| Monosomy 3 | 0 | 1** | 0 |

**Table 2:** Specific aneuploidies in descending order of frequency in first trimester losses as compared to 2nd and 3rd losses

* 5 mosaic for XX cell line/** 1 mosaic for normal cell line

## RESULTS (CONTINUED)

Chromosome analysis ordered on 186 samples

- 52 did not yield sufficient metaphase cells for analysis
- 26 yielded a normal male karyotype
- 8 samples showed both XX and XY cells (3 with abnormal male results also included in 22 abnormals)
- 81 yielded a normal female karyotype
  - MCC studies and concordant CMA results showed 27% of the 81 female results were truly normal female.
    - Data consistent with well known problem of maternal cell overgrowth of fetal cells in culture.
- 22 yielded abnormal results
  - 9 gave information not obtained from the array results
    - Table 4 shows 5 cases for which cytogenetics provided the diagnosis or additional context to the CMA findings.
    - For 5 cases identified as Trisomy 21 or 13 by CMA, karyotypes ruled out Robertsonian translocations.

## TABLE 3

| Copy Number Variation | Size (Mb) | Association | Classification |
|---|---|---|---|
| arr 7q11.23(72772522-74133319)x1 | 1.361 | Williams Syndrome | Pathogenic |
| arr 17p12(14128550-15551647)x3 | 1.423 | CMT1A | Pathogenic |
| arr 17q12(34522398-36136177)x3 | 1.614 | 17q12 duplication syndrome | Pathogenic |
| arr 22q11.23(23747662-24991856)x3 | 1.244 | Variable phenotype | Pathogenic |
| arr 7q11.23(72772522-74128940)x3 | 1.356 | WBS Duplication | Pathogenic |
| arr 17q12(34522398-36214026)x3 mat | 1.692 | 17q12 duplication syndrome | Pathogenic |
| arr[GRCh37] Xq22.2(103031457_103172394)x2 mat | 0.141 | *PLP1* gene Pelizaeus-Merzbacher | Pathogenic |

**Table 3:** Selected cases with clinically significant CNVs that are unlikely to result in pregnancy loss but are of significant clinical utility

## TABLE 4

| Cytogenetic Result | CMA Result | Indication | Discussion |
|---|---|---|---|
| 92,XXYY/46,XY | Normal Male | Severe growth restriction | 79% of cells tetraploid; CMA does not detect tetraploidy |
| 92,XXYY,t(7;13)(p15;q14)x2[16]/46,XX[4] | Normal male | Blighted ovum | Tetraploid cells in two cultures; possible paternal translocation if XX cells are maternal; CMA showed <5% MCC |
| 46,XY,t(2;22)(q12;q12) | Normal Male | IUFD | Possible parental translocation |
| 47,XY,+14[20] | Normal Male | Missed AB | Different pieces of tissue used for each method |
| 47,XY,+18[26]/46,XY[5] | Normal male | Missed AB | Different pieces of tissue used for each method |

**Table 4:** Cases in which chromosome analysis provided diagnostic information after a normal CMA

## DISCUSSION

- Chromosomal microarray analysis of DNA from direct extractions provides rapid, accurate diagnostic information on the etiology of pregnancy losses in any trimester without the classic problems incurred in cell culture
  - MCC studies allow assessment of the origin of normal female results, i.e., fetal or maternal
  - CMA may also provide information on pathogenic CNVs that may be of clinical utility to the family
- G-banded chromosome analysis, perhaps as reflex test, provides diagnostic information in some cases