Microarray-Based Cytogenetic Testing Offers Insights into the Genetic Underpinnings of Recurrent Pregnancy Loss

High-resolution analysis using the Infinium® CytoSNP-850K BeadChip provides high analytical sensitivity and specificity in identifying chromosomal and genomic abnormalities compared to conventional cytogenetic analysis methods.

Introduction

Miscarriage, or the loss of a pregnancy, is more common than many people realize. The American College of Obstetricians and Gynecologists (ACOG) estimates that approximately 1 in 5 clinically confirmed pregnancies will end spontaneously, most within the first 12 weeks of gestation.[1] What isn't as common is recurrent pregnancy loss (RPL), which the American Society for Reproductive Medicine (ASRM) defines as 2 or more miscarriages before those pregnancies (clinically confirmed by ultrasound) reach the 20-week mark.[2] Approximately 1–2% of women will suffer from multiple, consecutive miscarriages and, often, the cause of those losses is genetic in nature.[3]

According to Trilochan Sahoo, MD, Vice President of Clinical Affairs and Director of Cytogenetics at CombiMatrix, traditional chromosome karyotyping offers limited information about what might be causing RPL in certain couples. His longstanding work in constitutional cytogenetics and genomics suggested that chromosomal microarray analysis (CMA) might offer better reliability, analytical sensitivity, and specificity than older technologies for miscarriage analysis.[4-6] To test his hypothesis, Dr. Sahoo and his colleagues performed a > 3-year systematic, large-scale retrospective study.[7] The study unequivocally demonstrated that CMA using the CytoSNP-850K BeadChip helped detect clinically significant abnormalities in various sample types, offering more comprehensive information about chromosomal status than traditional karyotyping.

As a major provider of cytogenomic services, CombiMatrix performs cytogenetic analyses of more than 2500 samples from products of conception (POC) each year. Dr. Sahoo spoke with iCommunity about the historical challenges of using conventional chromosome analysis to understand the genetic underpinnings of RPL, and how CMAs like the CytoSNP-850K BeadChip will enable scientists and clinicians to gain critical insight into the causes of pregnancy loss.

Q: What is the incidence of miscarriage and RPL in the population?

Trilochan Sahoo (TS): Miscarriage is fairly common. It is accepted that about 15–20% of all clinically recognized pregnancies eventually result in a miscarriage event.[1] However, many pregnancies are lost before they are even recognized as a pregnancy by a doctor. About 1–2% of couples will have RPL.[3] We perform cytogenetic analysis of POC samples to determine the genetic causes that might be responsible for RPL.

Q: What are the difficulties in performing cytogenetic analysis of POC samples?

TS: There are several issues in performing cytogenetic analysis of POC samples. Miscarriages are hard to predict and often occur away from a clinical setting where a POC sample can be obtained quickly. Whether a miscarriage happens at home or in the hospital, there is also significant variability in the quality of POC samples we obtain. This is true even for fresh POC samples. Often, the samples are suboptimal in quality and labs cannot successfully culture the fetal cells of the chorionic villi required for a conventional chromosome analysis or a fluorescence *in situ* hybridization (FISH) analysis.

Historically, the assay failure rate is 20–40% when we perform conventional chromosome analysis on POC samples.[8-9] When we are successful, we run the risk of the sample being contaminated with maternal tissue. Maternal cell contamination with uterine tissue can make cytogenetic analysis of the POC sample not achievable and substantially increases the possibility of false negative results. Our best results come from DNA extracted purely and directly from the fetal tissue, thus providing the high-quality and highly specific data we need to understand its genomic status.

Trilochan Sahoo, MD, is Vice President of Clinical Affairs and Director of Cytogenetics at CombiMatrix.

Q: What led you to begin using the CytoSNP-850K BeadChip for POC cytogenetic analysis?
TS: We've been using the CytoSNP-850K BeadChip since 2012 exclusively for all of our cytogenomic testing, including prenatal, pediatric, and miscarriage analysis. It is a whole-genome, high-resolution microarray that detects abnormalities with high sensitivity and specificity. When we see a deletion or duplication of a chromosomal region using this microarray, we are confident it is accurate and true.

The CytoSNP-850K BeadChip evaluates 850,000 single nucleotide polymorphisms (SNPs), enabling us to identify important classes of genetic causes behind RPL. These include aneuploidies such as trisomies, triploidy where there are 3 complete haploid sets, and molar pregnancies, where an egg devoid of genetic material is fertilized by a sperm. Most of these abnormalities are not compatible with life and can be difficult to determine with conventional chromosomal analysis.

A valuable aspect of the CytoSNP-850K BeadChip is that it can be successfully used for formalin-fixed, paraffin-embedded (FFPE) tissue samples that have been processed in the pathology lab. We are able to analyze these FFPE samples, as well as fresh POC samples, with a high success rate. In fact, we can provide accurate and informative results in over 90% of fresh and FFPE samples we receive for microarray analysis using the CytoSNP-850K BeadChip. The ability to analyze FFPE samples is a benefit in researching multiple pregnancy losses, because we can go back to the archived POC samples from earlier pregnancies and determine what the abnormalities were.

> "We can provide accurate and informative results in over 90% of fresh and FFPE samples we receive for microarray analysis using the CytoSNP-850K BeadChip."

Q: What inspired your pregnancy loss sample study using CMA?
TS: CombiMatrix has been performing microarray analysis on POC samples for several years and is one of the largest providers of POC CMA services. With our implementation of the CytoSNP-850K BeadChip, we realized how powerful this technology was in identifying the standard trisomies that have been classically identified by chromosomal analysis, as well as a spectrum of additional abnormalities that are beyond the capabilities of traditional techniques. The CytoSNP-850K BeadChip can be used to identify a long list of abnormalities that might contribute to pregnancy loss. Because we had so much data, in numerous cases with many frequency findings, we felt it was incumbent upon us to make it available to the community.

We are proud to have published the largest single study of over 8000 POC samples using the latest generation of genomic tools. These new technologies are making a significant contribution to our understanding of the genetic causes of pregnancy loss and giving us the opportunity to ask questions about what else we need to know.

Q: What types of POC samples did you analyze in the CMA study?
TS: About 75% of the samples in our study were fresh samples, approximately 23% were FFPE samples, and a small percentage were blood, amniotic fluid culture cells, or DNA. Close to 60% were from patients with RPL.

> "We feel strongly that we have established a new standard for thinking about chromosomal aneuploidies and genomic imbalances resulting in, or contributing to, pregnancy loss."

Q: What technologies did you use in the CMA study?
TS: When we began testing POC samples several years ago, we used older CMA platforms, such as bacterial artificial chromosome "(BAC)" array comparative genomic hybridization (CGH) or oligonucleotide array CGH. The significance of our move to the SNP-based CytoSNP-850K BeadChip is especially true for FFPE samples, where chromosomal karyotyping is not possible and older platforms have a high failure rate. In our study, those technologies accounted for about 18% of cases. We transitioned to the CytoSNP-850K BeadChip in 2012, resulting in 88% of the study cases using this technology.

Q: What types of abnormalities were you assessing?
TS: When you look at POC samples, you commonly identify trisomy, triploidy, or large genomic imbalances such as deletions and duplications. Because of the size of our study, we obtained extensive data about the frequency of single chromosomal trisomy, such as trisomy 16 or trisomy 21. We also obtained a significant percentage of cases with multiple trisomies, where a patient had trisomy 16, plus trisomy 21 and another trisomy. Close to 8% had triploidy or whole-genome uniparental disomy (UPD). Triploidies are suggestive of partial hydatidiform moles and whole-genome uniparental disomy are indicative of a complete molar pregnancy; both these genomic abnormalities almost invariably result in pregnancy loss. Importantly, this large data set allowed us to get an accurate estimate of the frequency of specific chromosome abnormalities. We feel strongly that we have established a new standard for thinking about chromosomal aneuploidies and genomic imbalances resulting in, or contributing to, pregnancy loss.

Q: What insights can be gained using the CytoSNP-850K BeadChip to assess RPL?
TS: As you might imagine, patients who are suffering from RPL are driven to find answers as to why this is happening to them. Despite the commonness of miscarriage, pregnancy loss is not always easy to understand. The most recent studies suggest that genetic causes are the reason for more than 50% of pregnancy losses.[10-11] Yet, there still isn't much motivation to perform genetic testing to understand those losses.

The benefit of CMA testing is that we can, in more than 50% of the cases, actually define the abnormality. We can provide an answer that this is trisomy 21 or trisomy 16, or some other major genomic abnormality that resulted in this pregnancy loss. We now have a good idea about what degree and what type of genomic abnormalities are most capable of resulting in a pregnancy loss. We can't be as sure when the abnormalities are smaller and less obvious but, usually, we can say that a certain genetic abnormality caused or significantly contributed to a pregnancy loss.

The result is that we can provide patients with an answer. In many ways, it can give some sense of closure to this emotional and traumatic family event. In addition to that sense of closure, we can often provide some predictive value to the possibility of similar events occurring in future pregnancies. From our perspective, that might be the most valuable bit of information. It provides patients with information about their pregnancy loss, as well as what recurrence risk should be expected in future pregnancies.

> "The efficient workflow, the ability to process multiple samples simultaneously in a streamlined fashion, and the high-quality data we obtain with the CytoSNP-850K BeadChip gives us a high degree of confidence in making calls on the different types of abnormalities found in our samples."

Q: What is your assessment of the CytoSNP-850K BeadChip in analyzing POC samples?
TS: The CytoSNP-850K BeadChip is a powerful platform, providing us with genetic abnormality data with high sensitivity and specificity. It involves a streamlined process and user-friendly technology, from processing the samples to actually performing the microarray run and analyzing the data. The CytoSNP-850K BeadChip gives us the ability to work with a high degree of efficiency when processing multiple samples of various types. Much of the process is automated by technologies provided by Illumina and with processes that we developed in our lab. Most importantly, the CytoSNP-850K BeadChip gives us high-quality data, both for copy number variation, ie deletions, duplications, trisomies, and monosomies, and allelic heterogeneity. The efficient workflow, the ability to process multiple samples simultaneously in a streamlined fashion, and the high-quality data we obtain with the CytoSNP-850K BeadChip gives us a high degree of confidence in making calls on the different types of abnormalities found in our samples.

Q: How will you be using Illumina technology in the future?
TS: The CytoSNP-850K BeadChip is our preferred platform for analyzing POC for invasive prenatal testing and for assessing pediatric tissue samples.

Microarray technology, despite everything, is not as economical as we'd like it to be. So, we are assessing Illumina high-throughput, next-generation sequencing (NGS) technology for different fields of investigation. We are evaluating NGS to see if we can achieve the same degree of sensitivity and specificity that we have achieved using the CytoSNP-850K BeadChip. As we elaborated in our paper, if we can achieve some degree of assessment capability using NGS, we would be extremely proactive in pursuing it as a screening test before moving to CMA analysis for challenging POC samples.

The sensitivity, specificity, and abnormality pick-up rate provided by the CytoSNP-850K BeadChip will be a sort of gold standard for the immediate future. High-resolution chromosomal microarray offers immense value to our patients and their families, which is of primary importance to us.

Q: What do you hope findings will change regarding the study of RPL?
TS: There are still a substantial number of clinicians who believe conventional chromosomal karyotype analysis is the preferred choice for POC samples. In addition to our paper, there are other studies that have shown chromosomal microarrays, using the right platform, provide more answers with extremely high sensitivity and specificity.[6,12] That profoundly overrides anything that can be closely achieved by standard chromosome analysis. We hope to see an increasing number of clinicians and professional societies moving towards adopting this as the first line for POC testing in the future. We feel strongly that the patient receives significant benefit in terms of diagnostic value.

Not approved indication or product for this specific use. This opinion is based on the evaluation of the reviewer.

Learn more about the Illumina product mentioned in this article:

CytoSNP-850K BeadChip, www.illumina.com/products/by-type/clinical-research-products/infinium-cytosnp-850k.html

References

1. American Congress of Obstetricians and Gynecologists Practice Bulletin, Number 150, May 2015. www.acog.org/Resources-And-Publications/Practice-Bulletins/Committee-on-Practice-Bulletins-Gynecology/Early-Pregnancy-Loss. Accessed November 9, 2016.
2. American Society for Reproductive Medicine. What is recurrent pregnancy loss (RPL)? www.asrm.org/FACTSHEET_What_is_recurrent_pregnancy_loss/. Accessed November 9, 2016
3. Ford HB and Schust DJ. Recurrent Pregnancy Loss: Etiology, Diagnosis, and Therapy. *Rev Obstet Gynecol.* 2009; 2(2): 76-83.

4. Sahoo T, Cheung SW, Ward P, et al. Prenatal diagnosis of chromosomal abnormalities using array-based comparative genomic hybridization. *Genet Med.* 2016; 8(11): 719-727.
5. Shao L, Shaw CA, Lu XY, et al. Identification of chromosome abnormalities in subtelomeric regions by microarray analysis: a study of 5,380 cases. *Am J Med Genet A.* 2008; 146A(17): 2242-2251.
6. Wang BT, Chong TP, Boyar FZ, et al. Abnormalities in spontaneous abortions detected by G-banding and chromosomal microarray analysis (CMA) at a national reference laboratory. *Mol Cytogenet*. 2014; doi: 10.1186/1755-8166-7-33.
7. Sahoo T, Dzidic N, Strecker MN, et al. Comprehensive genetic analysis of pregnancy loss by chromosomal microarrays: outcomes, benefits and challenges. *Genet Med.* 2016;. doi: 10.1038/gim.2016.69.
8. Bell KA, Van Deerlin PG, Haddad BR, et al. Cytogenetic diagnosis of "normal 46,XX" karyotypes in spontaneous abortions frequently may be misleading. *Fertil Steril.* 1999; 71(2): 334-341.
9. Reddy UM, Page GP, Saade GR, et al. Karyotype versus microarray testing for genetic abnormalities after stillbirth. *N Engl J Med.* 2012; 367(23): 2185-2193.
10. Menasha J, Levy B, Hirschhorn K, et al. Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. *Genet Med.* 2005; 7(4): 251-263.
11. Stephenson M and Kutteh W. Evaluation and management of recurrent early pregnancy loss. *Clin Obstet Gynecol.* 2007; 50(1): 132-145
12. Levy B, Sigurjonsson S, Pettersen B, et al. Genomic imbalance in products of conception: single-nucleotide polymorphism chromosomal microarray analysis. *Obstet Gynecol.* 2014: 124(2 Pt 1): 202-209.

Illumina, Inc. • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

illumina®