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Analysis of tissue from products of conception and perinatal losses using QF-PCR and microarray: A three-year retrospective study resulting in an efficient protocol

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ABSTRACT

Objective: To evaluate the performance of a laboratory protocol for direct genetic analysis performed on tissues obtained from miscarriages, stillbirth and postnatal death.
Methods: Samples were collected between July 1st, 2011 and June 30th, 2014. QF-PCR analysis was the initial test followed by aCGH analysis performed on the normal QF-PCR specimens.
Results: Of the 1195 submitted specimens, a total of 1071 samples were confirmed as true fetal. The failure rate was 1.4%. Of those, 30.8% yielded abnormal results. Of the latter, 57.6% had abnormal QF-PCR and 42.4% had abnormal microarray result. Autosomal trisomies were detected in 61.2%, triploidy in 7.6%, monosomy X in 9.1%, sex-chromosome aneuploidy (apart from monosomy X) in 1.5%, molar pregnancies in 5.8% and copy number variants in 14.2% including microdeletions/microduplications and cryptic unbalanced rearrangements. The highest diagnostic yield was observed in the 1st trimester specimens at 67.6%. We confirmed that maternal age correlates with the likelihood of autosomal trisomies but not with triploidy, sex chromosome aneuploidies, molar pregnancy, or CNVs.
Conclusion: An efficient laboratory protocol, based on QF-PCR and aCGH of uncultured cells has replaced standard cytogenetic analysis in testing of tissue from all pregnancy losses in our center and resulted in reduced test failure rate and increased diagnostic yield.

1. Introduction

Despite much improvement in preconception and prenatal care over the past decades, women continue to face the risks of miscarriages and mid-trimester stillbirths. Approximately 1/5 of all first-trimester recognized pregnancies result in miscarriages and stillbirth. Stillbirth is defined as fetal loss after 20 weeks of gestation and occurs at a frequency of 1 in 165 births in the United States (MacDorman and Kirmeyer, 2009). Although miscarriages and perinatal losses are etiologically a heterogeneous condition and include maternal, fetal or placental abnormalities, chromosomal abnormalities are by far the most common cause found in up to 50% of first trimester losses (Hassold et al., 1980; Gardner et al., 2012). Efforts in investigating early and late fetal losses have reduced the number of cases of unknown etiology; but one quarter to half of stillbirths remained unexplained (Smith and Fretts, 2007). Determining the pregnancy losses' karyotype has a major impact on the woman/couple's future reproductive plans in that it distinguishes between non-familial and familial chromosomal abnormalities and detects gestational trophoblastic disease to help in post-loss follow-up.

Traditionally, karyotyping has been used for genetic testing of perinatal losses; however, this technique requires actively dividing live cells and led to several challenges, such as culture failure from nonviable pregnancies, poor quality sample, maternal cell contamination, as well as microbial contamination resulting in

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culture failure. In the past few years, microarray has replaced conventional karyotyping in an attempt to solve some of these issues. In a population-based study of 532 stillbirths conducted by the Stillbirth Collaborative Research Network, traditional karyotyping was compared to microarray testing for the diagnosis of chromosome abnormalities in stillbirth (Reddy et al., 2012). The authors showed that microarray analysis has a greater success rate in providing results, 87.4% of the cases compared to 70.5% in karyotype analysis, and provided better detection of aneuploidy and pathogenic copy-number variants (8.3% vs. 5.8%), mainly due to its success in testing non-viable tissue. Another more recent study on 481 consecutive stillbirth cases by Sahlin et al. also concluded that microarray analysis has a significantly higher success in the detection rate compared to conventional karyotyping (Sahlin et al., 2014). Other laboratory techniques have been used for the analysis of fetal losses including fluorescence *in situ* hybridization (FISH), multiplex-ligation dependent probe amplification (MLPA) and quantitative fluorescent PCR (QF-PCR) alone (Jobanputra et al., 2002; Bruno et al., 2006; Diego-Alvarez et al., 2005, 2007). However, these techniques provide information limited to only the targeted common aneuploidies.

In a previous paper, we had proposed a laboratory protocol for direct genetic analysis on uncultured cells of tissue obtained from miscarriages and perinatal losses using QF-PCR followed by aCGH in normal QF-PCR results (Morgen et al., 2012). From here on, this will be referred as the uncultured protocol. We have shown that this protocol not only detects all abnormal cases diagnosed by standard karyotyping but also identifies new pathogenic copy-number variants otherwise undetectable by conventional G-banding. This protocol not only resulted in a better diagnostic yield but also reduced the cost and improved turnaround time. Therefore, at our institution, we have adopted this uncultured protocol for all solid tissue specimens from products of conception and perinatal losses.

The objective of this retrospective study is to evaluate the performance of this protocol used on tissues obtained from miscarriages, stillbirths and postnatal demise during the past three-year period at our institution.

2. Materials

All samples included in the current study consisted of tissue from products of conception and perinatal losses that were submitted to the Cytogenetics Laboratory at Mount Sinai Hospital, a large tertiary care center and teaching hospital, from July 1st, 2011 to June 30th, 2014 for a total of three years. Only solid tissues were included such as skin, umbilical cord, cartilage and products of conception (POC) from autopsies. Analysis of all tissue samples by the uncultured protocol was performed under the routine and extensive quality control/assurance programs of the diagnostic laboratory, following the Canadian College of Medical Geneticists (CCMG) guidelines regarding the handling of residual specimens after diagnosis (CCMG, 2008). The study was performed in accordance with the rules and regulations of the Mount Sinai Hospital's research ethics board.

3. Methods

We used the SoftMolecular module (SCC Soft Computer Inc) laboratory information system (LIS) to retrieve all tissue specimens from products of conception and perinatal losses submitted between July 1st, 2011 and June 30th, 2014 for a total study period of three years.

3.1. Uncultured protocol

DNA was obtained from uncultured specimens by proteinase K digestion, followed by phenol/chloroform extraction using a manual Phase Lock Gel (PLG) Heavy System (5 Prime, Hamburg, Germany) and ethanol precipitation. Quantity and quality of DNA was evaluated by 260, 280 and 230 nm absorbance measurements (NanoDrop Technologies, Inc., USA), and the integrity of high-molecular-weight genomic DNA was assessed by electrophoresis on a 0.8% agarose gel stained with ethidium bromide. The majority of specimens yielded high-quality DNA, and only 15 of the 1086 cases were excluded from the study due to poor sample quality and DNA degradation.

3.2. QF-PCR analysis

QF-PCR analysis was performed on all DNA specimens to assess the presence of common aneuploidies using an Aneufast QF-PCR kit according to the manufacturer's instructions (Genomed Diagnostics AG, Switzerland). The QF-PCR/Aneufast assay contains 19 highly polymorphic Short Tandem Repeats (STR) markers on chromosomes 13, 18, 21, X and Y, and 2 non-polymorphic markers on chromosomes X and Y, reflex testing. PCR products were analyzed on an ABI 3130*xl* Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Interpretation of results was performed using guidelines from the Aneufast manual, the 2007 ACC/CMGS "QF-PCR for the diagnosis of aneuploidy best practice guidelines" V2.01, and the CCMG "Practice Guidelines for Prenatal QF-PCR". Maternal blood DNA was also analyzed and compared to any accompanying placental villi specimens with a normal female QF-PCR result, to ensure that the sample was solely conceptus and not contaminated by cells of maternal origin. Maternal cell contamination (MCC) was determined by calculating the proportion of the secondary profile from the primary profile using the peak areas of informative STR markers. At least 7 informative markers were required to establish fetal/maternal origin of the analyzed profiles. Abnormalities detected by QF-PCR resulted in final reporting without follow-up by aCGH.

3.3. Array-CGH analysis (aCGH)

After QF-PCR analysis, only specimens with normal or uninformative results continued to aCGH analysis. Array CGH was performed on test DNA using an 8 × 60K ISCA v2.0 (AMADID 26370) oligonucleotide array (BlueGnome Ltd., UK) according to the CytoChip Oligo™ protocol (BlueGnome Ltd., UK). This is a commercially available, whole-genome ISCA-designed oligonucleotide array with a median resolution of 150 kb and tiling coverage in known clinically-relevant regions (496 targeted regions with a mean probe spacing of 3.5–4.6 kb). The sex-matched reference DNA was derived from pooled peripheral blood leukocytes donated by phenotypically normal individuals (Promega, Madison, WI, USA). Following the protocol, 500 ng–1 μg of tissue sample DNA and its corresponding 500 ng-1ug of sex-matched reference DNA were independently labelled and co-hybridized to the 8 × 60K ISCA v2.0 oligonucleotide array. Next, post-hybridization washes were performed using a Little Dipper Microarray Processor (SciGene, Sunnyvale, CA, USA) according to the CytoChip BlueGnome protocol, and the arrays were scanned using an Agilent G2565CA microarray scanner system (Agilent Technologies, Santa Clara, CA, USA). The resulting data was analyzed using the BlueFuse Multi software package (BlueGnome Ltd., UK).

All detected copy number variants (CNV) were systematically evaluated following recommendations from the American College of Medical Genetics (ACMG) guidelines (Kearney et al., 2011).