Embracing the challenges of isochromosomes in cell-free DNA prenatal screening

Theresa Boomer, Erica Soster, Jason Chibuk, Samantha Caldwell, Eyad Almasri, Jenna Wardrop, Sida Boshes, Michelle Hackbardt, Philip Cacheris, Ron McCullough
Integrated Genetics, *Sequenom*Inc., Laboratory Corporation of America

I. Introduction

Cell-free DNA (cfDNA) prenatal screening continues to increase insight into early gestational placental findings not previously recognized or well understood. More recently, genome-wide sequencing combined with advanced bioinformatics has enabled even more detailed interrogation of a variety of complex chromosomal changes, including isochromosome formation.

We previously presented a small series of isochromosome cases screened via cfDNA and noted several unique biologic caveats and potential limitations of detection with cfDNA and diagnostic testing alike. In relation, other laboratories have noted an over-representation of false-negative cfDNA results among cases with isochromosome formation. Herein we provide an updated review our laboratory experience over the last 7 years, including a wide variety of isochromosomal rearrangements.

II. Methods

Maternal blood samples were submitted for either MaterniT® 21 PLUS or MaterniT® GENOME cfDNA screening, subjected to DNA extraction, library preparation, and genome-wide massively parallel sequence as described by Jensen et al.1 Depending on the assay ordered, sequencing data were analyzed using novel algorithms as described by Zhao et al. and Leffowitz et al.2

Contingent on the particular test ordered, the scope of chromosomal anomalies reviewed and reported on differ greatly. For instance, a MaterniT® 21 PLUS test opting for just the ‘core aneuploidies’ is inclusive of Trisomy 13/18/21 only. Anomalies outside of the three aneuploidies are by design not routinely flagged for lab director review, and thus typically unknown and unreported. A provider may opt to assessment of sex chromosome anomalies and/or an expanded panel including a select list of 7 well-defined microdeletions, along with trisomies 16 and 22. Meanwhile, the MaterniT® GENOME test utilizes a greater depth of sequencing, is inclusive of genome-wide aneuploidy detectors, as well as select microdeletions and subchromosomal copy number variants (CNVs) ≥ 7Mb, all of which are flagged and routinely reviewed by a genetic counselor and lab director prior to reporting. Regardless of the test initially chosen, all of our screening tests employ genome-wide genome sequencing and thus retrospective review of genome-wide raw data for MaterniT® 21 PLUS is possible and occasionally requested by providers, albeit without the increased sequencing depth provided by the MaterniT® GENOME test. This study cohort includes a mixture of both prospectively reported chromosome anomalies, as well as those only noted upon retrospective review post provider request. All outcome data (cytogenetic/molecular testing results) are recorded in our internal clinical database.

IV. Discussion & Conclusions

Isochromosomes are often accompanied by mosaicism, as structural abnormalities incite mitotic instability.3 There are a variety of mechanisms that lead to isochromosome formation, with the “classic mode” depicted in Figure 4. This error in centromere division can occur during both meiosis or mitosis, and selective growth advantage of the normal cell line is common.

The variable nature of isochromosome formation (meiotic vs. mitotic error), along with inherent mosaicism, poses significant biologic limitations for cfDNA screening technology and subsequent diagnostic testing alike. Early placenta cytotrophoblast, as reflected in cfDNA, may be concordant or discordant with later maternal blood testing as well. Depending on the assay ordered, reported result may or may not reflect abnormal cfDNA test data.

The emergence of genome-wide cfDNA sequencing has allowed greater insight and understanding of the isochromosome phenomenon. While the established biologic limitations of isochromosome detection remain unavoidable, genome-wide cfDNA screening promises to be an additional, effective tool for early identification of many isochromosomes that repeatedly occurred throughout the genome.

Key points:

• The most common isochromosome ‘detected’ via cfDNA are i(Y)p, i(Y)q, i(12p), i(13q), and i(18q).

• Down Syndrome associated with i(21q) carries a relatively high risk of false negative cfDNA results.

• The majority of fetal isochromosome cases produce abnormal cfDNA trace data and yield abnormal test results.

• Underlying complex biology of isochromosome formation confounds cfDNA screening and diagnostic testing alike.

V. References


2. Huisken van Amstel und et al. Isochromosome 21p is overrepresented among false-negative cell-free DNA prenatal screening results involving Down syndrome. European Journal of Human Genetics. 2015:10.1038/ejhg.2015.80. doi:10.1038/ejhg.2015.80. 2015 Mar:


