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1. Sequenom Laboratories

## I. Introduction

Noninvasive prenatal testing (NIPT) for aneuploidy relies on the presence of circulating cell free DNA believed to be largely placental in origin. The genetic material in fetal and placental tissue matches in most pregnancies. However, discordance between these tissues can result from post zygotic non-disjunction or trisomy rescue, causing uneven distribution of cells between fetus and placenta. This is not a new limitation of NIPT technology.<sup>1,2</sup> Five recently confirmed cases of confined placental mosaicism (CPM) identified by positive NIPT results are highlighted here.

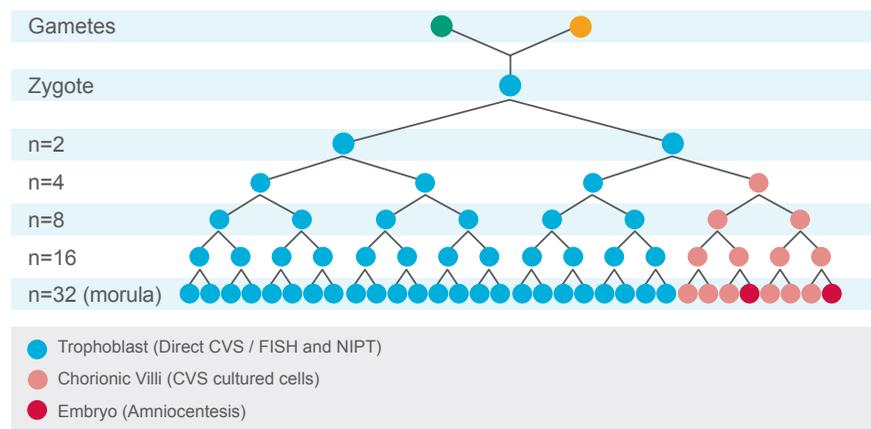
## III. Results

Figure 1. Five cases of confirmed CPM

Cases	1	2	3	4	5
NIPT Ordered	MaterniT 21 PLUS	MaterniT GENOME	MaterniT 21 PLUS	MaterniT 21 PLUS	MaterniT GENOME
GA at NIPT Draw	12 weeks 1 day	10 weeks 4 days	10 weeks 5 days	10 weeks 0 days	11 weeks 6 days
Indication for NIPT	AMA	Per/Fam Hx	AMA	AMA	AMA
NIPT Result	Trisomy 21	18p dup; 11q del	XO	Trisomy 21	18p dup
CVS FISH results	Trisomy 21	NA	NA	NA	T18
CVS Karyotype results	Mosaic Trisomy 21	18p dup; 11q del mosaic	35% XO	64% Trisomy 21	Normal
CVS Array results	NA	18p dup; 11q del mosaic	NA	NA	Extra material 18p mosaic
Amnio FISH results	NA	NA	NA	NA	Normal
Amnio Karyotype results	Normal	Normal	Normal	Normal	NA
Amnio Array results	NA	Normal	NA	NA	Normal

Figure 2. Early cell lineage post conception

- The majority of cells develop into placental trophoblast / chorionic ectoderm (Direct CVS preparation & NIPT)
- A small minority of cells develop into chorionic villi / mesoderm (CVS cultured cells)
- Only two cells go on to form the embryo and amniotic tissues (Amniocentesis)



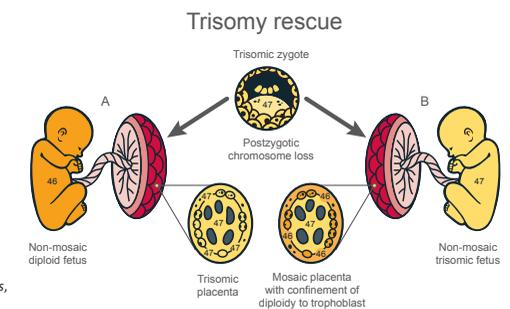
Thomas, D et al. (1994, July 10) *Trisomy 22, placenta*. Retrieved from <https://www.sonoworld.com/Fetus/page.aspx?id=182>.

## II. Methods

Maternal blood samples submitted to Sequenom Laboratories® for MaterniT® 21 PLUS and MaterniT® GENOME testing were subjected to DNA extraction, library preparation, and whole genome massively parallel sequencing as described by Jensen et al.<sup>3,4</sup>

Figure 3. Post-zygotic rescue of a trisomic conceptus

- A. A Non-mosaic diploid fetus with a trisomic (likely mosaic) placenta complement as a product of mitotic loss of the trisomic chromosome in the embryonic progenitor cells.
- B. Non-mosaic trisomic fetus with a mosaic placenta complement and diploid trophoblast as a product of early postzygotic mitotic loss of the trisomic chromosome.



Kaloupek, D. K., & Vekemans, M. (1996). Confined placental mosaicism. *Journal of Medical Genetics*, 33(7), 529-533.

## IV. Conclusions

These cases describe five pregnancies with positive NIPT results, negative amniocentesis, and confirmed CPM. These cases underscore how NIPT provides unprecedented insight into the chromosomal constitution of placental tissue.

CPM that would be otherwise unseen or minimized by traditional test methods confounds results both technically and clinically. NIPT mirrors short term CVS cultures (including FISH), both of which primarily examine the trophoblastic layer of the placenta. Trophoblasts are especially prone to mitotic error due to the rapid cell division necessary for uterine wall attachment. Early differentiation of this placental layer makes it the most distantly related cell type compared to the relatively few cells that go on to form the fetus proper. Long term CVS cultures examine a mixture of trophoblast and villus core cells, the latter being more closely related to the fetus proper. Amniotic fluid samples contain cells from the epiblast of the inner cell mass of the embryo and most closely reflect the true constitution of the fetus.

Long term cell culture can bias analysis, since culture conditions favor normal, healthier cell lines and select against abnormal clones. Thus, analysis of mosaic cultured cells is likely to be an underrepresentation of the abnormal cells that existed *in vivo*.

CPM may be associated with varying degrees of placental dysfunction, and may present clinically as abnormal biochemical screening, fetal growth restriction, or preterm labor. Fetal growth restriction is directly associated with certain types of CPM. Specific CPM types are indistinguishable by NIPT and difficult to assess prenatally as a whole. CPM types I and III are indistinguishable by NIPT, while type II may allude NIPT altogether. Therefore, pregnancies that present with discordant 'false positive' NIPT results may warrant more conservative pregnancy management, as they may be at risk for placental dysfunction. Clinicians need to be aware of the various biological limitations of each testing modality, as well as cell culture impact on mosaic studies. Especially in the face of discrepant prenatal results, such awareness is essential for accurate clinical assessment and counseling and overall case interpretation.

## V. References

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- Lefkowitz RB, Tynan J, Liu T, et al. Clinical validation of a non-invasive prenatal test for genome-wide detection of fetal copy number variants *American Journal of Obstetrics & Gynecology*. doi:http://dx.doi.org/10.1016/j.ajog.2016.02.030.