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Laboratory experience reporting trisomy 16 and 22 from cell-free DNA screening: Diagnostic and clinical outcome data over a four-year period

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I. Objectives

Cell-free DNA (cfDNA) screening for trisomy 16 (T16) and trisomy 22 (T22) has been clinically available as part of the MaterniT® 21 PLUS laboratory-developed test since October 2013. In 2016, early laboratory screening experience for these two esoteric trisomies was presented.[1] Updated outcome data are described here.

II. Methods

Maternal blood samples submitted for MaterniT®21 PLUS testing were subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing as previously described by Jensen et al.[2] Data analysis was performed on reportable cases for which trisomy 16 and 22 screening was ordered as part of the Enhanced Sequencing Series (ESS). For positive results, outcome data were elicited by phone or email from the ordering provider.

III. Results

OVERVIEW

During the study period, we identified 200 cases positive for trisomy 16, and 163 positive for trisomy 22. Sixty percent (n=120) of positive T16 cases, and 45% (n=74) of positive T22 cases had follow-up information (e.g. diagnostic testing and/or obstetric outcome information) reported to the laboratory. A flowchart of pregnancy outcome data can be seen in **Figure 1**.

Overall, 81% (97/120) of the positive trisomy 16 cases and 78% (58/74) of the positive trisomy 22 cases with outcome information showed concordance with, or a suspected biological explanation for, the cfDNA finding (i.e. confirmed by prenatal diagnosis, documented co-twin demise, or pregnancy complications suggestive of CPM, covert fetal mosaicism, or uniparental disomy).

Figure 1. Flowchart of cases positive for Trisomy 16 and Trisomy 22

* Follow-up information: results of diagnostic testing and/or obstetric outcome information

** Diagnostic testing: karyotype and/or microarray analysis via CVS, amniocentesis, products of conception, or postnatal blood

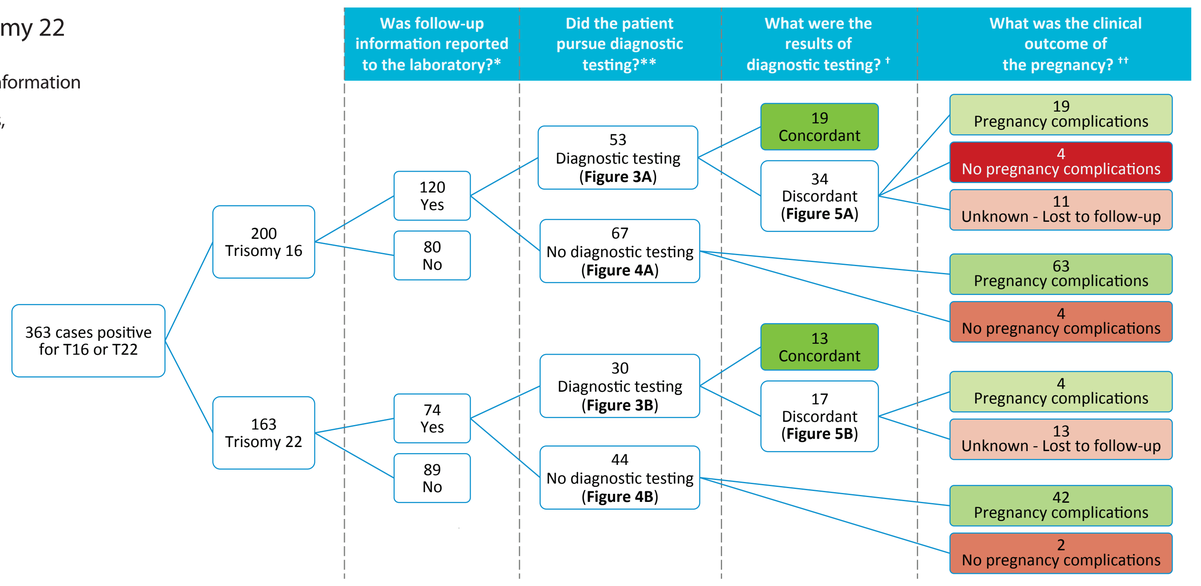
† Concordant: karyotype and/or microarray results were consistent with the cfDNA finding

Discordant: karyotype and/or microarray results did not confirm the cfDNA finding

††Pregnancy complications suggestive of confined placental mosaicism, true/cryptic fetal mosaicism, or uniparental disomy, included: fetal/co-twin demise, IUGR, preterm delivery, preeclampsia, and ultrasound abnormalities

NOTE:
Data shown in shades of green are cases in which the diagnostic and/or clinical outcome of the pregnancy were concordant (to varying degrees) with the cfDNA result.

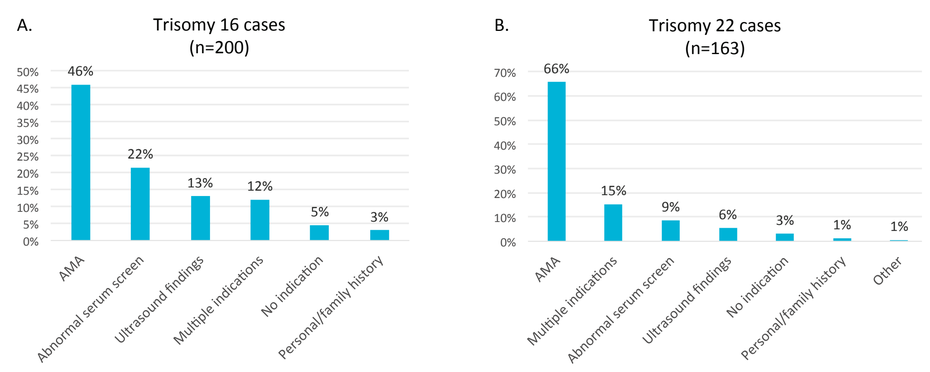
Data show in shades of red are cases in which the diagnostic and/or clinical outcome of the pregnancy were discordant with the cfDNA results.



INDICATION FOR TESTING

Of the overall cohort (T16 n=200; T22 n=163), the majority of screen positive samples were from patients referred for testing due to advanced maternal age. Cases positive for trisomy 16 were more frequently referred for abnormal serum screening results (22%) compared to cases positive for trisomy 22 (9%).

Figure 2. Indication for testing

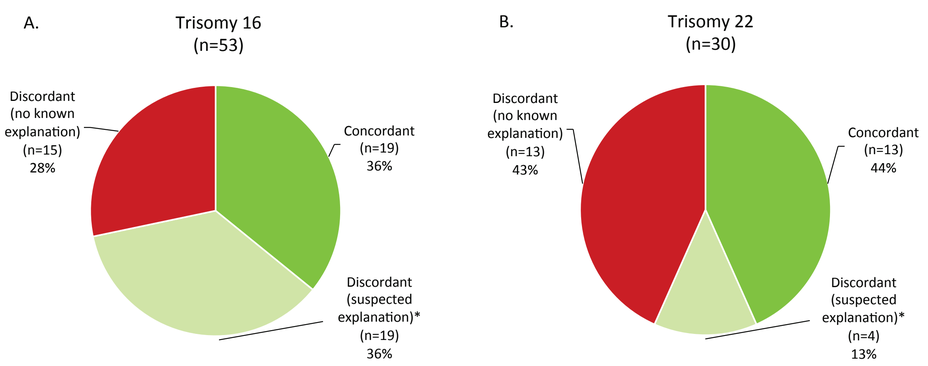


TRISOMY 16

Of the 120 positive trisomy 16 cases with follow-up information reported to the laboratory, 44% (n=53) had diagnostic testing (i.e. karyotype and/or microarray analysis on chorionic villus sampling, amniocentesis, products of conception, or peripheral blood). Diagnostic results confirmed the NIPT finding in 36% of these cases (n=19/53). 64% of diagnostic results were discordant with the NIPT findings (n=34/53). (**Figure 3A**) Upon review of these 34 discordant cases, 19 reported pregnancy complications (e.g. IUGR, preterm delivery, ultrasound abnormalities, etc.) suggestive of confined placental mosaicism (CPM), covert fetal mosaicism, or uniparental disomy (UPD). The remaining 15 cases had normal pregnancy outcomes (i.e. none of the above-mentioned complications were reported) or were lost to follow-up. A detailed review of the clinical outcome data for discordant T16 results can be seen in **Figure 5A**.

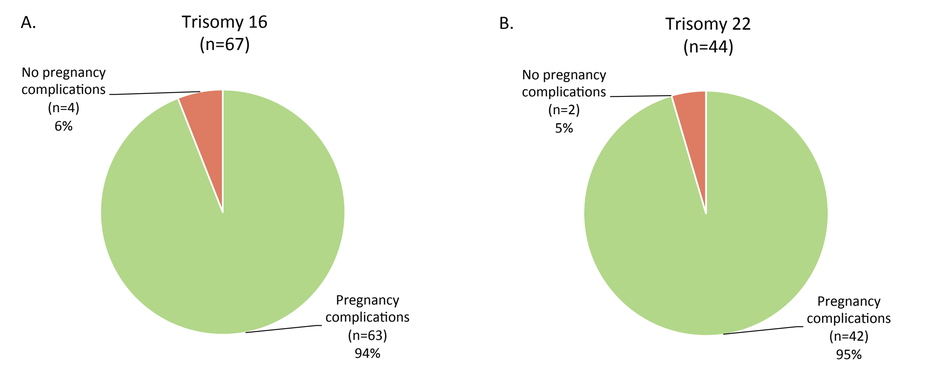
There were 67 cases in which the patient declined diagnostic testing, but clinical information was reported to our laboratory regarding the outcome of the pregnancy. Sixty-three of these 67 pregnancies (94%) had pregnancy complications to support a likely biological explanation for the abnormal NIPT result. Four cases had normal pregnancy outcomes. (**Figure 4A**)

Figure 3. Outcomes for cases with clinical feedback and diagnostic testing



*Suspected explanation:
Reported pregnancy complications from providers (e.g. IUGR, preterm delivery, ultrasound abnormalities, etc.) suggestive of confined placental mosaicism (CPM), covert fetal mosaicism, or uniparental disomy (UPD)

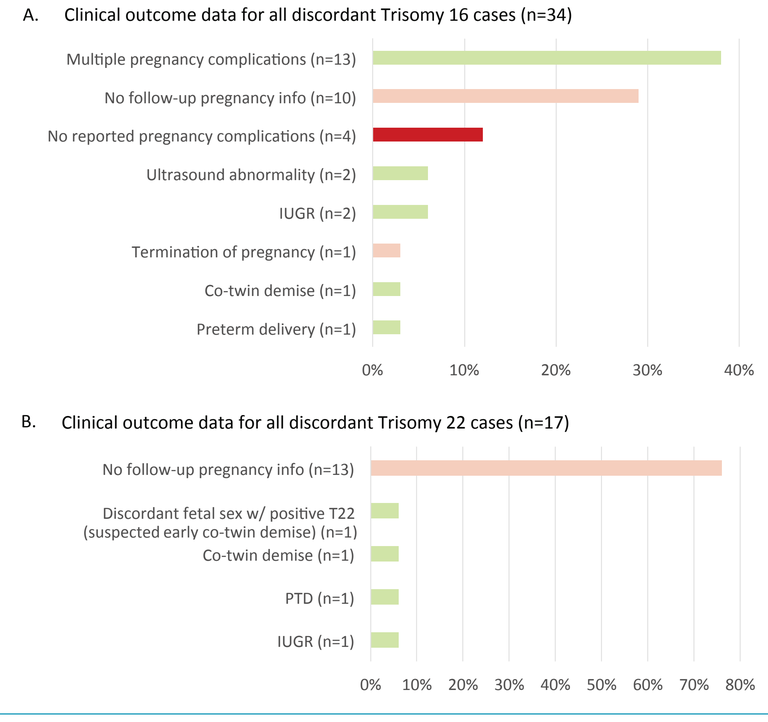
Figure 4. Outcomes for cases with clinician feedback, but no diagnostic testing



TRISOMY 22

Of the 74 positive trisomy 22 cases with follow-up information reported to the laboratory, 41% (n=30) had diagnostic testing, of which 43% of results were concordant with the NIPT findings (n=13). The remaining 57% of cases (n=17) were discordant, and only 3 of the discordant cases had reported pregnancy complications. (**Figure 3B**) A detailed review of the clinical outcome data for discordant T22 results can be seen in **Figure 5B**.
Of cases with no diagnostic follow-up (n=44), 95% had pregnancy complications to support the abnormal NIPT result (n=42). (**Figure 4B**)

Figure 5. Breakdown of pregnancy outcomes for cases in which diagnostic testing results were discordant with the cfDNA findings



IV. Conclusions

Though T16 and T22 are regarded as "common causes of miscarriage" and thus unlikely to affect an ongoing pregnancy, there is increasing evidence to suggest that esoteric aneuploidies can have significant implications for the health of a pregnancy. The fetus may exhibit ultrasound abnormalities due to true fetal mosaicism or uniparental disomy resulting from a trisomy rescue event. The pregnancy may develop complications such as IUGR, preeclampsia, preterm delivery, or miscarriage due to an abnormal placenta that is mosaic for aneuploidy. Furthermore, the abnormal cfDNA results may provide an explanation for the demise of a co-twin. Given these points, T16 and T22 should be considered appropriate conditions for inclusion on cfDNA screening panels.

Key points:

- Genome-wide cell-free DNA screening may identify pregnancies with esoteric aneuploidies
- In our testing cohort, 83 patients (23%) pursued diagnostic testing after receiving a positive cfDNA result for trisomy 16 or 22, and results were confirmed in 32 of these cases (39%)
- Even in the absence of diagnostic confirmation of the cfDNA finding, pregnancy complications (i.e. IUGR, preeclampsia, preterm delivery, miscarriage) were common
- Overall, 81% of the positive trisomy 16 cases, and 78% of the positive trisomy 22 cases with outcome information showed concordance with, or a suspected biological explanation for, the cfDNA finding

V. References

1. Boomer T, et al. Discordant noninvasive prenatal testing (NIPT) results and placental health: A blessing in disguise. Poster presented at: *ACMG Annual Clinical Genetics Meeting*. 2016 Mar 8-12; Tampa, FL.
2. Jensen TJ, Zwiefelhofer T, Tim RC, et al. High-throughput massively parallel sequencing for fetal aneuploidy detection from maternal plasma. *PLOS One*. 2013; 8(3):e57381. doi:10.1371/journal.pone.0057381. Epub 2013 Mar 6.

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