

# Esoteric aneuploidies and genome-wide cell-free DNA

B-209

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## I. Introduction

Cell-free DNA (cfDNA) testing for common aneuploidies has been integrated into prenatal care for both high-risk and average-risk pregnancies. Expansion of cfDNA technology includes select microdeletion syndromes, large copy number variants, and esoteric aneuploidies. Such esoteric aneuploidies are most likely to be confined to the placenta, but fetal involvement cannot be ruled out without further testing. In a large published study of over 50,000 chorionic villus samples (CVS), Grati et al. showed that approximately 1.81% (1136/52,673) of cases suggested mosaicism. In cases with a follow-up karyotype by amniocentesis (n=886), a fetal chromosome abnormality was found in 12.8% of cases.<sup>1</sup> In the remainder, the mosaicism appeared to be confined to the placenta. While confined placental mosaicism (CPM) has been observed with normal pregnancy outcomes, literature suggests that pregnancies with CPM are at risk for adverse outcomes related to placental insufficiency including growth restriction and preterm labor.<sup>2,3,4</sup> Because cfDNA is believed to be derived from the trophoblast, cfDNA provides a unique non-invasive opportunity for assessment of the placenta. We describe the trends seen in the initial clinical data regarding pregnancies that screened positive for esoteric aneuploidies on a genome-wide cfDNA test.

## II. Methods

A retrospective analysis was performed on over 28,000 maternal blood samples submitted for MaterniT® GENOME genome-wide cfDNA analysis at Sequenom® Laboratories. Samples were subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing.<sup>5</sup> Sequencing data were analyzed using a novel algorithm to detect aneuploidies and sub chromosomal, genome-wide events 7Mb and larger.<sup>6</sup> The results reported as positive for at least one esoteric aneuploidy (excluding chromosomes 21, 18, 13, X and Y) were separated and reviewed. Clinical outcomes were requested from ordering providers as part of routine follow-up of all positive samples.

## III. Results

In over 28,000 samples, there were 1392 results reported as positive. Approximately 14% (n=197) screened positive for any esoteric aneuploidy (excluding 21, 18, 13, X and Y) (Figure 1). While the vast majority of positive esoteric aneuploidy cases were positive for a single aneuploidy, there were 9 cases positive for multiple autosomal aneuploidies. Those 9 cases were excluded from this analysis, resulting in 188 cases that screened positive for a single autosomal aneuploidy. Of these cases, 36.7% (n=69) had outcome data available (i.e. final pregnancy outcome with delivery/birth information), 27.1% (n=51) had outcome information available (i.e. CVS or amniocentesis results, but not final pregnancy outcome), and 36.2% (n=68) cases were either still ongoing pregnancies at the time of data analysis or were lost to follow-up.

Advanced maternal age was the single most common indication in this cohort (Figure 2). In order of frequency, the most common positive result for an esoteric aneuploidy involved chromosomes 7, 16, 22, 3, and 15, collectively accounting for over half of the cases (Figure 3).

Figure 4 describes the adverse outcomes in this cohort of cases positive for a single esoteric aneuploidy. Overall, at least 44.7% of cases (84/188 as delineated by the red boxes) had an adverse pregnancy outcome, defined as growth restriction, preterm labor, pregnancy loss/fetal demise, or structural ultrasound anomalies. Removing the 19 pregnancies with confirmed fetal or placental events, 41.4% (70/169) of the remaining pregnancies had an adverse pregnancy outcome.

### DISTRIBUTION OF POSITIVE RESULTS

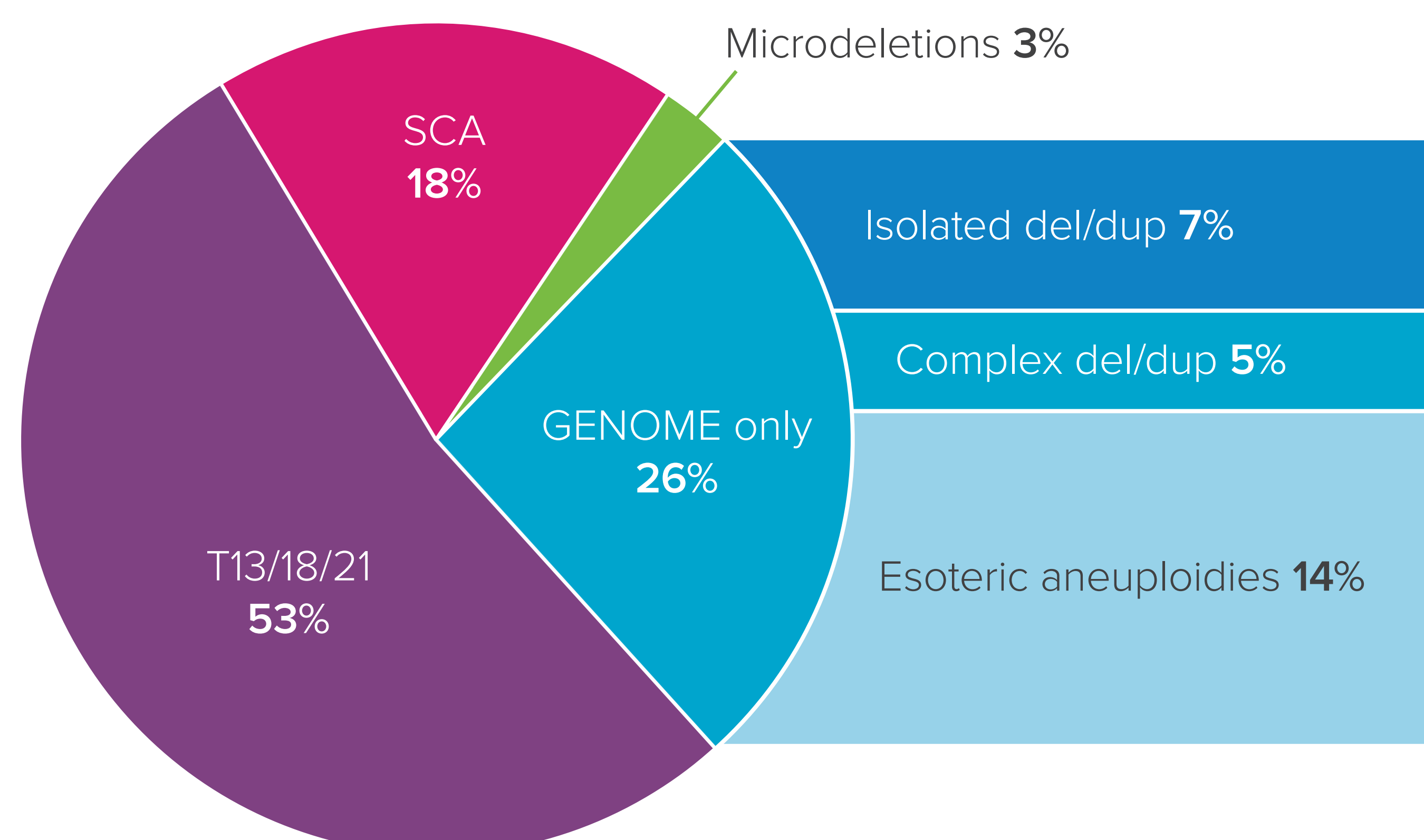


Figure 1: All positive cases (n=1392 of >28,000 total samples)

### REASON FOR REFERRAL

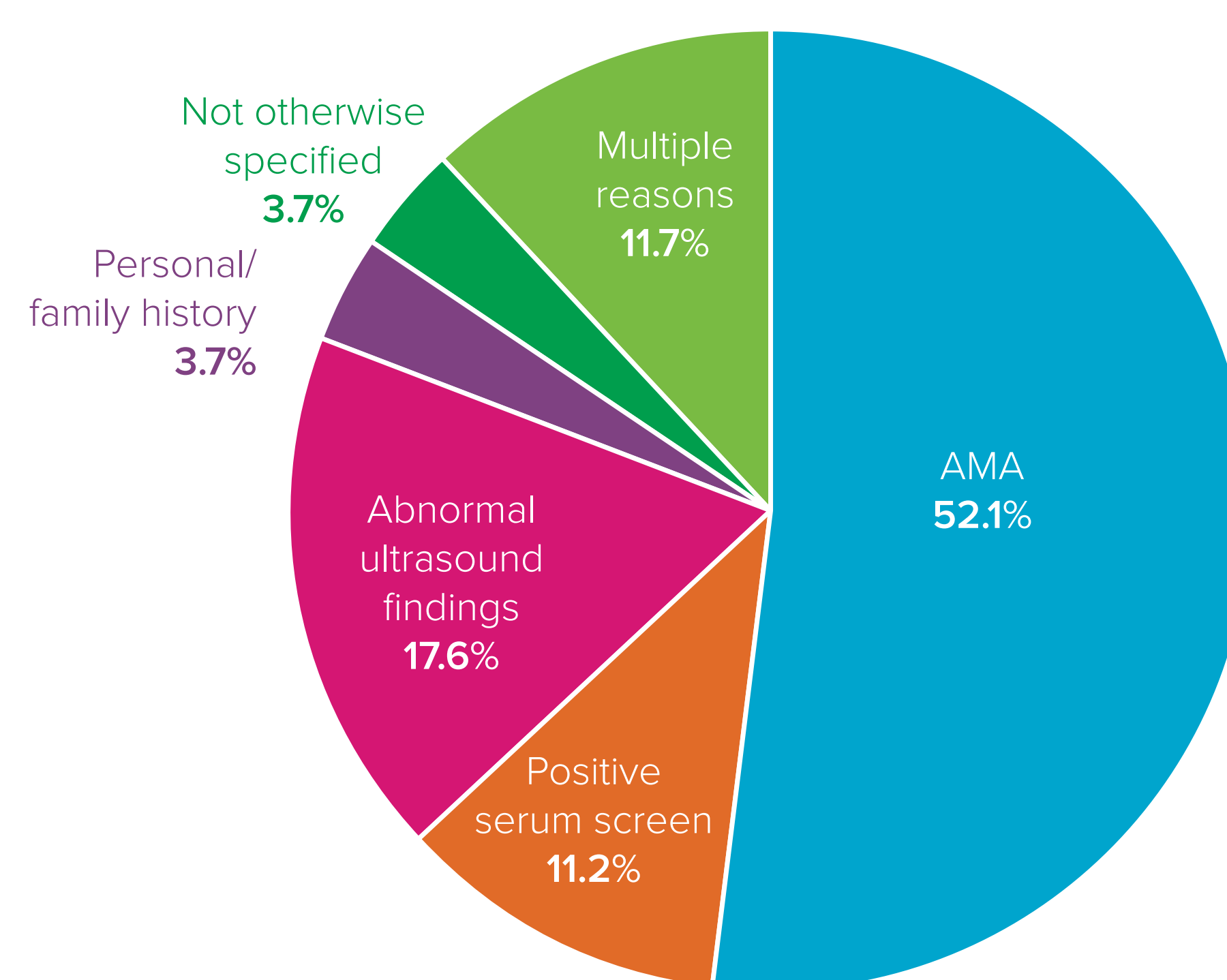


Figure 2: Distribution of reason for referral among cases positive for a single esoteric aneuploidy (n=188)

### NUMBER OF POSITIVE CASES PER CHROMOSOME

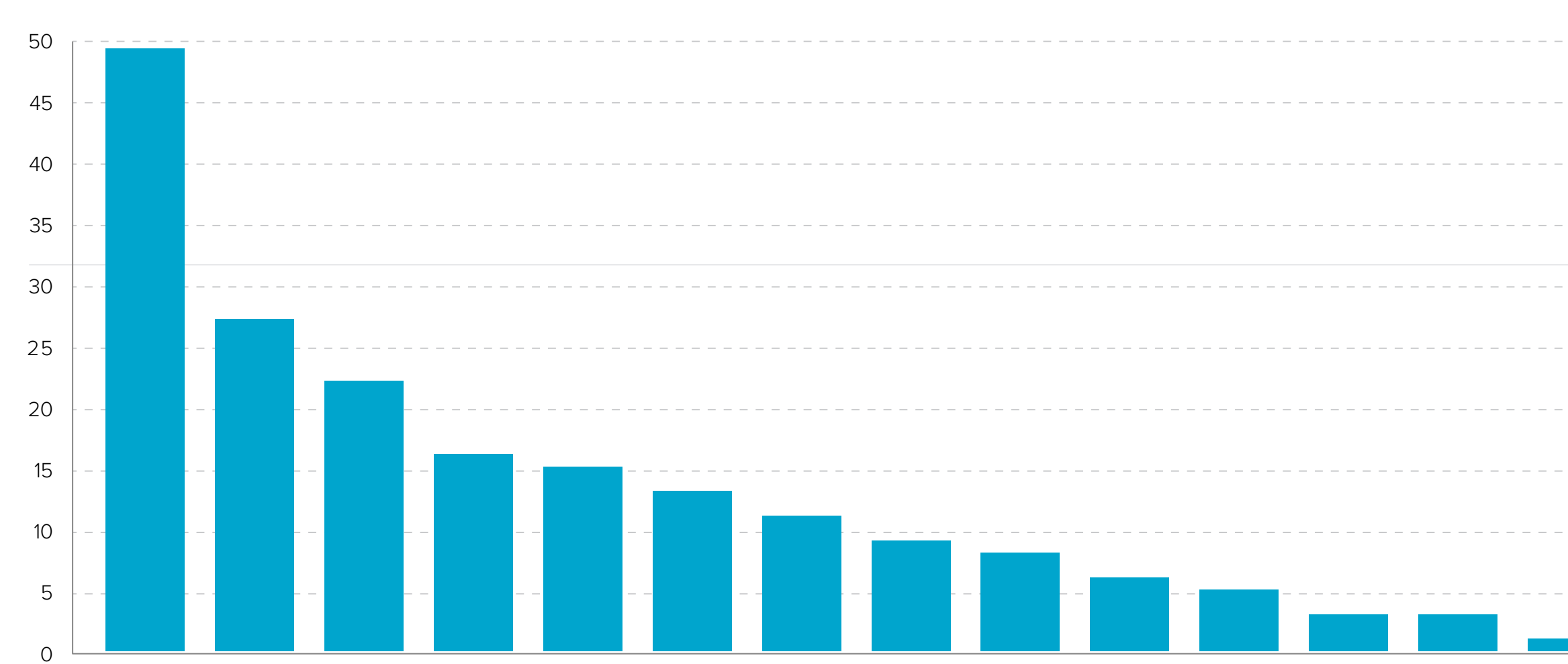


Figure 3: Number of cases reported positive by the laboratory for an esoteric aneuploidy, by chromosome. For chromosomes 5, 6, 12, 17, and 19 there were no reported cases.

Outcome	Percent of cases (n=188)
Confirmed event in fetus or placenta <sup>a</sup>	10.1% (19/188)
Suspected placental event (likely CPM) or fetal event <sup>b</sup>	3.2% (6/188)
Miscarriage/fetal demise without testing	10.1% (19/188)
All discordant cases <sup>c</sup>	30.3% (57/188)
• Discordant with adverse outcome <sup>d</sup>	49/1% (28/57)
Ongoing pregnancy or lost to follow-up	46.3% (87/188)
• Ongoing/lost to follow-up with adverse outcome <sup>d</sup>	19/5% (17/87)

Table 1: Outcomes of cases positive for a single esoteric aneuploidy based on provider feedback

(a) Includes full trisomy, mosaicism, loss of heterozygosity, or UPD. (b) Cases were considered suspected placental/fetal event when clinical information included growth restriction, preterm labor, or structural ultrasound anomalies but diagnostic testing was not performed. (c) Cases in which any diagnostic testing returned normal results. (d) Adverse pregnancy outcome is defined as growth restriction, preterm labor, pregnancy loss/fetal demise, or structural ultrasound anomalies.

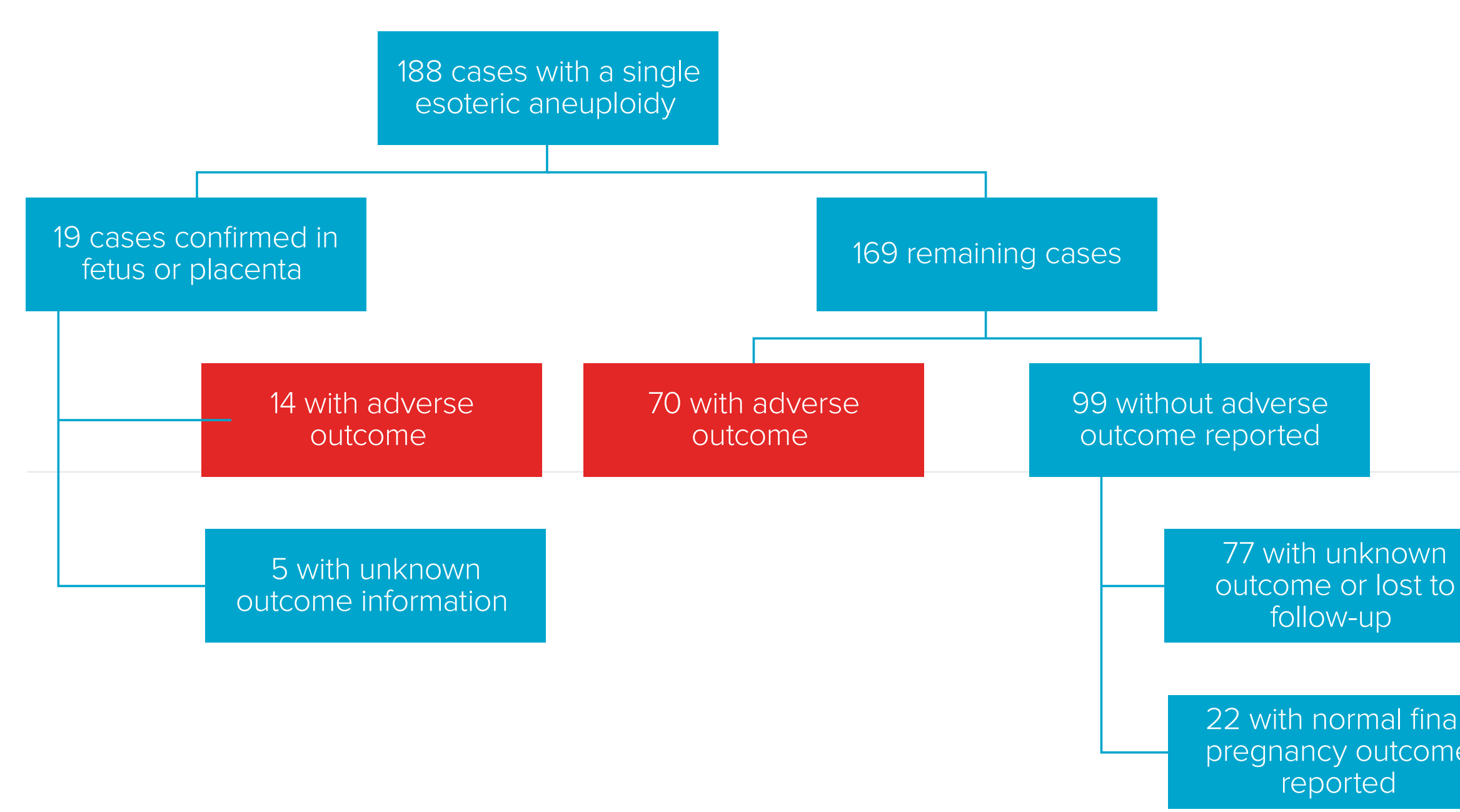


Figure 4: Adverse pregnancy outcomes are defined as growth restriction, preterm labor, pregnancy loss/fetal demise, or structural ultrasound anomalies.

## IV. Discussion

Based on the early clinical outcome results, nearly half of all cases that were positive for an esoteric aneuploidy on MaterniT® GENOME test had a clinically relevant outcome including growth restriction, preterm labor, pregnancy loss/fetal demise, or structural ultrasound anomalies. Some cases of confirmed fetal or placental events were lost to follow-up or the pregnancies were terminated before development of pregnancy complications could be assessed.

One of the significant analytical limitations of the discordant cases is the type of diagnostic testing performed. Only 13.2% of discordant cases included any sort of placental testing (such as CVS or postnatal placental studies). In the remainder of cases, placental mosaicism could not be ruled out and adverse pregnancy outcomes (including growth restriction or ultrasound findings) might be considered suggestive of placental involvement. Given the placental origin of cfDNA, these cases may be discordant for fetal status, but the trisomic event may still exist in the placenta and thus is “real” to the cfDNA platform. Another important point to consider in all cases is the possibility of long-term culture bias against abnormal cell lines or tissue-specific mosaicism which cannot be excluded when only one tissue type is interrogated, such as has been reported to occur with many of the rare autosomal aneuploidies.<sup>7</sup>

Additional considerations in the discordant cases include other biological limitations of cfDNA screening. In 18.9% of the discordant cases a biological limitation, such as a twin demise or suspected maternal event, was identified as the likely explanation of the discordant result.

Future research may include investigating the clinical utility of a positive cfDNA result for esoteric aneuploidies and whether certain aneuploidies are more likely to result in a benign outcome or conversely result in a poor outcome. A more robust sample size of positive cases with outcomes is required for such analysis and is also a limitation of the current analysis. Furthermore, exploration into the implications of multiple aneuploidy cases is warranted, but was not currently feasible given a limited number of cases with limited follow-up data available at this time. Cooperation from the ordering providers in sharing diagnostic testing results and clinical outcomes is necessary for the laboratories to collect clinical outcome data, and is often a limitation of relying on ad hoc provider feedback. The current data set was limited by having complete follow-up in less than 50% of the cases, although many had partial follow-up.

Ultimately, counseling on a positive cfDNA result for an esoteric aneuploidy may be complex, but the results hold potentially valuable clinical utility and the range of possible outcomes must be considered. Residual risk for fetal mosaicism/aneuploidy, confined placental mosaicism, uniparental disomy, and adverse pregnancy outcome exist after a positive screen for an esoteric aneuploidy. All of these outcomes have been represented in this cohort. As such, discordant results should not be dismissed without consideration of these additional risks.

## V. Conclusion

- Out of over 28,000 MaterniT® GENOME cases, 1392 were reported as positive. Of all positives, 14% were reported as positive for an esoteric aneuploidy.
- The most common single esoteric aneuploidies involved chromosomes 7, 16, 22, 3, and 15, collectively accounting for over half of the cases.
- Of all cases reported positive for an esoteric aneuploidy, 44.7% of cases were reported to have an adverse outcome.

## VI. References

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