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Size distribution of ‘True Positive’ 22q11.2 deletions by cfDNA

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1. Introduction

Since the advent of cfDNA screening for fetal chromosome abnormalities in 2011, many patients have been screened for ‘expanded content’ such as sex chromosome aneuploidies, rare autosomal aneuploidies, and copy number variants including microdeletions. When screening for fetal copy number variants by cfDNA, many factors will affect assay performance including event size, fetal fraction, sequencing depth, maternal CNVs, and regional variation. In general, smaller events at lower fetal fractions are more challenging to detect than larger events at higher fetal fractions. Microdeletion syndromes are caused by deletions of various sizes in the region of interest. Some cfDNA assays may only report on microdeletions over a certain size threshold, excluding detection of smaller, nested or atypical deletions. For 22q11.2 deletion syndrome, approximately 85% of patients have a ~2.54 Mb sized deletion, which had been frequently described as a ‘3 Mb’ deletion, from the A-D low copy number repeats (LCRs)[1]. The other ~15% have smaller atypical or nested deletions. This study examined the size distribution of 22q11.2 deletions from diagnostic testing following a positive cfDNA result at one laboratory.

2. Methods

A retrospective review of cfDNA results positive for 22q11.2 deletion syndrome (DS) from massively parallel sequencing was performed. Samples were submitted for either MaterniT®21 PLUS or MaterniT® GENOME testing and were subjected to DNA extraction, library preparation, and whole genome massively parallel sequencing as described by Jensen et al[2] and Lefkowitz et al[3]. Samples from 2013 and later were included, capturing positive results reported since microdeletion analysis became clinically available. Cases identified as true positive by confirmatory diagnostic testing with deletion size available were included. Cases without diagnostic testing, or without an available deletion size from testing (i.e. FISH), were excluded from analysis. Cases which only confirmed maternal events (either negative in the fetus or undetermined fetal status) were included if the size of the maternal deletion was available. Of note, since the launch of microdeletion screening at this laboratory, a size threshold for reportable abnormalities has not been imposed.

For most cases (37/45), mosaicism ratio values were available. Mosaicism ratio is a laboratory metric derived by dividing the fraction of cfDNA associated with the abnormal event by the overall fetal fraction of the specimen, as described by Rafalko et al.[4] Indications for testing are recorded as provided by the ordering clinician at the time of testing. For cases in which this metric were available, mosaicism ratios were compiled and analyzed.

Study data was statistically described using counts, rates, and measures of central tendency. Statistical analysis and generation of plots and figures was performed using R version 4.0.5 and the dplyr, ggplot2, and ggpubr packages[5-8]. When comparing the groups with and without ultrasound findings and gestational age at testing, normality of gestational age data was tested using a Shapiro-Wilk test and compared using a Mann-Whitney U test.

3. Results

Table 1 shows a summary of 22q11.2 deletion size ranges confirmed by diagnostic testing. All cases, including maternal and fetal confirmations are described. In general, maternal deletions were smaller than fetal deletions, with a median size of 0.7 Mb compared to 2.5Mb, respectively.

Figure 1 shows the distribution of deletion sizes confirmed on diagnostic testing. The different colors indicate whether the deletion was confirmed in the fetus, mother, or both, as denoted by the legend.

Figure 2 shows the distribution of Mosaicism Ratio (MR) of the confirmed cases, using the same color designation to display in whom the deletion was confirmed. Note the clear separation of cases: all fetal cases fell below an MR of 2, with all maternal events having an MR above 4.

Figure 3 shows the indications for testing. Fifteen cases were sent for screening due to ultrasound findings; another 6 cases in the ‘multiple indications’ group included ultrasound findings as one of the reasons for referral. In total, 46.7% (21/45) of cases were known to have ultrasound findings at the time of testing. For 16 cases (35.6%) no ultrasound findings were reported at the time of testing, while for 8 cases (17.8%) ultrasound information was unknown.

Figure 4 shows a comparison of the gestational age at testing between the group known to have ultrasound findings (n=21) compared to the group that did not have ultrasound findings (n=16) at the time of testing. Eight cases where this information was unknown were excluded. Cases with no ultrasound findings at the time of testing almost exclusively occurred before the 18th week of pregnancy, while cases with ultrasound findings almost always occurred after the 18th week of pregnancy; a statistically significant finding (p=8.868e-06).

4. Conclusions

A significant number of cases (~91%) had a deletion below 2.91 Mb, and 60% had a deletion measuring below 2.5 Mb. Of the fetal-confirmed cases, ~89% had a deletion below 2.91 Mb and ~48% had a smaller, nested or atypical deletion measuring below 2.5 Mb. Approximately 85% of patients with 22q11.2 DS have a ~2.54 Mb sized deletion, which had been frequently described as a ‘3 Mb’ deletion, from the A-D low copy number repeats (LCRs)[1] while the other ~15% have smaller atypical or nested deletions. Common FISH probes used by diagnostic laboratories (such as N25, TUPLE, and *TBX1*) hybridize in areas between the A-B LCRs, so deletions that do not include this region will not be ascertained using FISH alone[1].

Despite a relatively small sample size in this study, the data suggest a higher percentage of nested, atypical deletions than commonly reported, with the majority of cases with confirmed deletions smaller than the ‘common’ ~2.54 Mb deletion. Although smaller deletions are harder to detect, using a 2.91 Mb (or even 2.54 Mb) threshold for 22q11.2 DS would limit the sensitivity of a cfDNA assay, as deletions less than the designated threshold would return screen negative for a significant number of affected cases. Future studies could explore the size distribution for 22q11.2 DS on prenatal diagnostic testing to see if the same trends are true.

A significant portion of cases (46.7%) were known to have an ultrasound finding at the time of cfDNA testing. Although an imperfect assumption, one might presume that the other ~53% of cases did NOT have ultrasound findings at the time of cfDNA testing. Furthermore, the distribution of gestational ages between the group with ultrasound abnormalities and the group without was statistically significant. Cases without ultrasound anomalies were almost exclusively sent in the first trimester and early second trimester, before the time of routine anatomy scan. Patients alerted to an increased risk earlier in pregnancy presumably have additional time for decisions about diagnostic testing and pregnancy management, as compared to patients who do not have testing until after an ultrasound finding is identified. This information may be useful for providers and patients when deciding whether to opt-in to microdeletion screening, especially for 22q11.2 deletion syndrome, on routine cfDNA screening.

Lastly, as shown in **Figure 2**, mosaicism ratio may be a useful laboratory metric in predicting when a case is more likely to be maternal or fetal in origin. When the cfDNA sequencing data suggests a maternal CNV, the strong signal produced by the maternal event precludes assessment of fetal status for that chromosome region. This result typically does not affect interpretation of the rest of the genome, but if the maternal event is confirmed, the fetus is at 50% risk to inherit the CNV.

This data is limited by a small number of cases. Furthermore, the data represents the experience of a single laboratory collecting retrospective outcome data and may not be applicable to other assays. Cases only confirmed by FISH testing were excluded because deletion size from diagnostic testing was required for inclusion in this analysis. Therefore, this cohort may have some bias towards those smaller or atypical deletions since they may not be amenable to FISH confirmation, given the loci used for probe hybridization. This underscores the importance of microarray in cases where cfDNA suggests a microdeletion, particularly in the event of 22q11.2 deletion syndrome

Key Points:

- Confirmed 22q11.2 deletions ranged in size from 0.268 – 3.26 Mb.
- Patients with a positive 22q11.2 deletion result without ultrasound findings were more likely to be tested earlier in pregnancy, compared to those tested after an ultrasound finding was identified.
- Of patients with a confirmed 22q11.2 deletion, 60% had an identified nested, or atypical deletion smaller than 2.5 Mb. A minimum size threshold would limit the sensitivity of a cfDNA assay as smaller deletions below the cut-off would be screen negative for the deletion.

Tables + Figures

Table 1. Summary of size data for 22q11.2 deletions confirmed on diagnostic testing after a positive cfDNA screening result for 22q11.2 deletion syndrome.

	Range (Mb)	Median Size (Mb)	Mean Size (Mb)	Cases < 2.9 Mb	Cases < 2.5 Mb
All cases (n=45)	0.268 – 3.26	2.5	1.865	91.1% (41/45)	60.0% (27/45)
Maternal (n = 21)*	0.268 – 2.96	0.749	1.267	95.2 % (20/21)	81.0% (17/21)
Fetal (n = 27)*	0.433 – 3.26	2.540	2.195	88.9% (24/27)	48.1 (13/27)

*There were three cases confirmed in both the pregnant patient and the fetus.

Figure 1. Distribution of deletion sizes confirmed by diagnostic testing for 22q11.2 deletion syndrome following positive cfDNA screening.

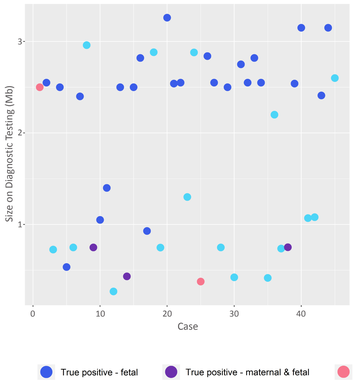


Figure 2. Distribution of mosaicism ratio of cases confirmed on diagnostic testing

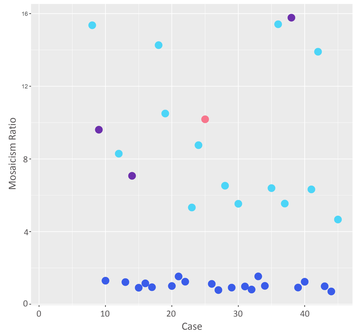
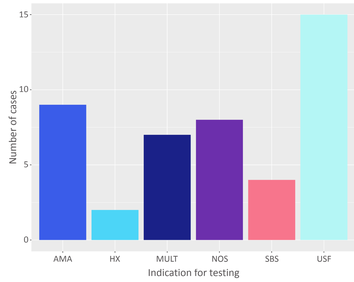
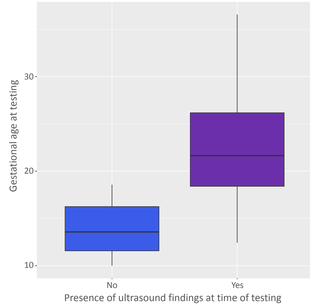


Figure 3. Indications for testing for the cohort.



AMA = Advanced maternal age, HX = Personal/Family History,
MULT = Multiple indications, NOS = Not otherwise specified,
SBS = Abnormal serum biochemical screening,
USF = Ultrasound finding(s)

Figure 4:.Comparison of gestational age at time of cfDNA testing for cases with and without ultrasound findings. The two groups are significantly different, p=8.868e-06.



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