

Referral indications and demographic trends observed in over 1,500 samples positive for sex chromosome aneuploidy on prenatal cell-free DNA screening

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1. Introduction

Cell-free DNA (cfDNA) prenatal screening for 45X, 47XXX, 47XXY, and 47XYY, referred to as sex chromosome aneuploidies (SCAs), has been clinically available since 2013. Collectively, the incidence of SCAs is 2-3X higher than the incidence of Trisomy 21 (T21) in patients <35 years; 1 in 294 compared to 1 in 1,250 – 1 in 714.¹ With increased ordering of cfDNA in average-risk patients even prior to recent ACOG support, this study aimed to analyze demographic and referral trends in a positive SCA-only cohort.^{1,2}

2. Methods

A retrospective review of 1,515 positive SCA-only results identified by cfDNA screening from one laboratory was performed. All samples were subjected to DNA extraction, library preparation, and genome-wide massively parallel sequencing.³ Indications for referral were provided by the ordering clinician on the test requisition form (TRF). 'Maternal age' includes specimens referred >35 years at the time of delivery while 'no known high risk' includes patients <35 years at the time of delivery. For these specimens, no additional high risk indications were provided by the clinician on the TRF. Specimens without a marked indication for referral were adjudicated to the maternal age or no known high risk category based on maternal age at the time of delivery.

Demographic and laboratory metrics (maternal age, gestational age, turnaround time, and fetal fraction) were described using counts, rates, and measures of central tendency. A statistical analysis to compare positive SCA results based on indication for referral, including a demographic comparison between 45X to the SCA trisomies (47XXX, 47XXY, and 47XYY) was performed. VassarStats was used to complete 2 sample, two-sided t tests to compare demographic/laboratory metrics. A Fisher's Exact Test and Chi Square analysis were performed using R 4.0.5 for comparison of referral indications.⁴ A p value <0.05 was considered statistically significant.

3. Results

Average maternal age, gestational age, fetal fraction, and turnaround time for 45X, 47XXX, 47XXY and 47XYY are outlined in **Table 1**. The average maternal age and gestational age are statistically different when comparing positive 45X results to positive SCA trisomies. There is no statistical difference when comparing fetal fraction and turnaround time. (**Table 2**)

Figure 1 outlines positive SCA results based on common indications for referral including maternal age, no known high risk, ultrasound finding (USF), and other. 'Other' includes abnormal biochemical screening, multiple indications, personal/family history, and other less common or not well defined indications. A Fisher's Exact test shows a statistically significant association between the listed indication for referral and whether results are positive for 45X or one of the SCA trisomies (p value <0.001). In particular, positive 45X results are 3X more likely than SCA trisomies when USF is the only indication for referral. This 3-fold increase remains stable even when including multiple indications with USF. There is no statistical difference amongst SCA results when comparing maternal age and no known high risk indications (Fisher's Exact Test, p -value=0.06) and no statistical difference between referral indications amongst the individual SCA trisomies (Chi Square Analysis, p -value=0.3).

4. Conclusions

This study shows statistically significant demographic trends between positive SCA results by cfDNA screening. In particular, a lower average maternal age and gestational age was observed in patients positive for 45X. The higher incidence of SCAs compared to T21 in patients <35 years, combined with an average maternal age <35 years for SCA positive results, supports the utility of SCA screening in all patients and should be discussed in pre-test counseling.

There are statistically significant differences when looking at cfDNA referral indications and whether results will be positive for 45X or SCA trisomy. Some important limitations of this analysis is the indication for referral may not always be provided by the ordering clinician on the TRF and only reflects the information known at the time of testing. The 3-fold increase in positive 45X results over SCA trisomies when USF is the sole referral indication likely contributes to the statistical significance across all indications and result type.

Overall, the 3-fold increase in positive 45X results when USF is present combined with the lower gestational age of 12.9 weeks in the 45X cohort highlights the importance of early first trimester ultrasound. The average maternal age of 32.1 years for 45X combined with a lack of statistical significance when comparing age-only indications for referrals amongst positive SCA results supports prior literature suggesting age-related X chromosome loss is unlikely to influence cfDNA results for 45X.⁵ Further research can explore fetal and maternal (when applicable) diagnostic test results to analyze clinical test performance.

Key Points:

- The higher incidence of SCAs combined with an average maternal age <35 years for SCA positive results supports the utility of SCA screening in all patients.
- An average maternal age of 32.1 years for 45X and no statistical significance comparing age referral indications supports literature suggesting age-related X chromosome loss is unlikely to impact cfDNA results for 45X.
- The 3-fold increase in 45X results when USF is present and the average earlier gestational age of 12.9 weeks in 45X highlights the importance of early first trimester ultrasound.

Tables + Figures

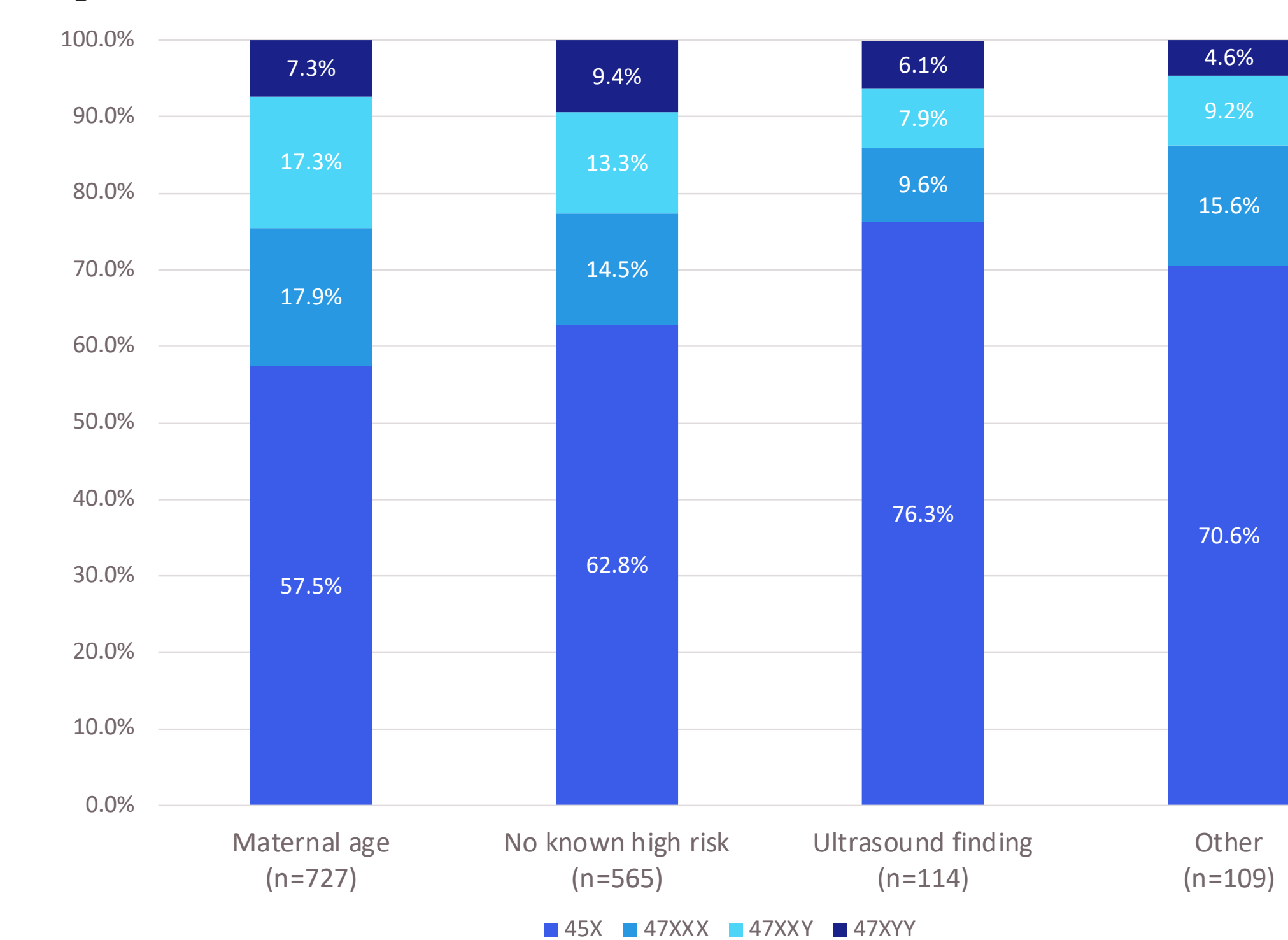
Table 1. Demographic information of a positive SCA-only cohort

Average	45X (n=937)	47XXX (n=240)	47XXY (n=220)	47XYY (n=118)
Maternal age (years)	32.1	33.2	34.1	32.4
Gestational age (weeks)	12.9	13.6	13.5	13.4
Fetal fraction	8.4%	7.7%	8.8%	9.1%
Turnaround time (days)	4	4	3	4

Table 2. Demographic comparisons between 45X and SCA trisomies

Average	45X (n=937)	SCA Trisomies (n=578)	p-value
Maternal age	32.1 years	33.4 years	<0.001
Gestational age	12.9 weeks	13.6 weeks	0.006
Fetal fraction	8.4%	8.4%	0.826
Turnaround time	4 days	4 days	0.139

Figure 1. Positive SCA result based on indications for referral



References

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