

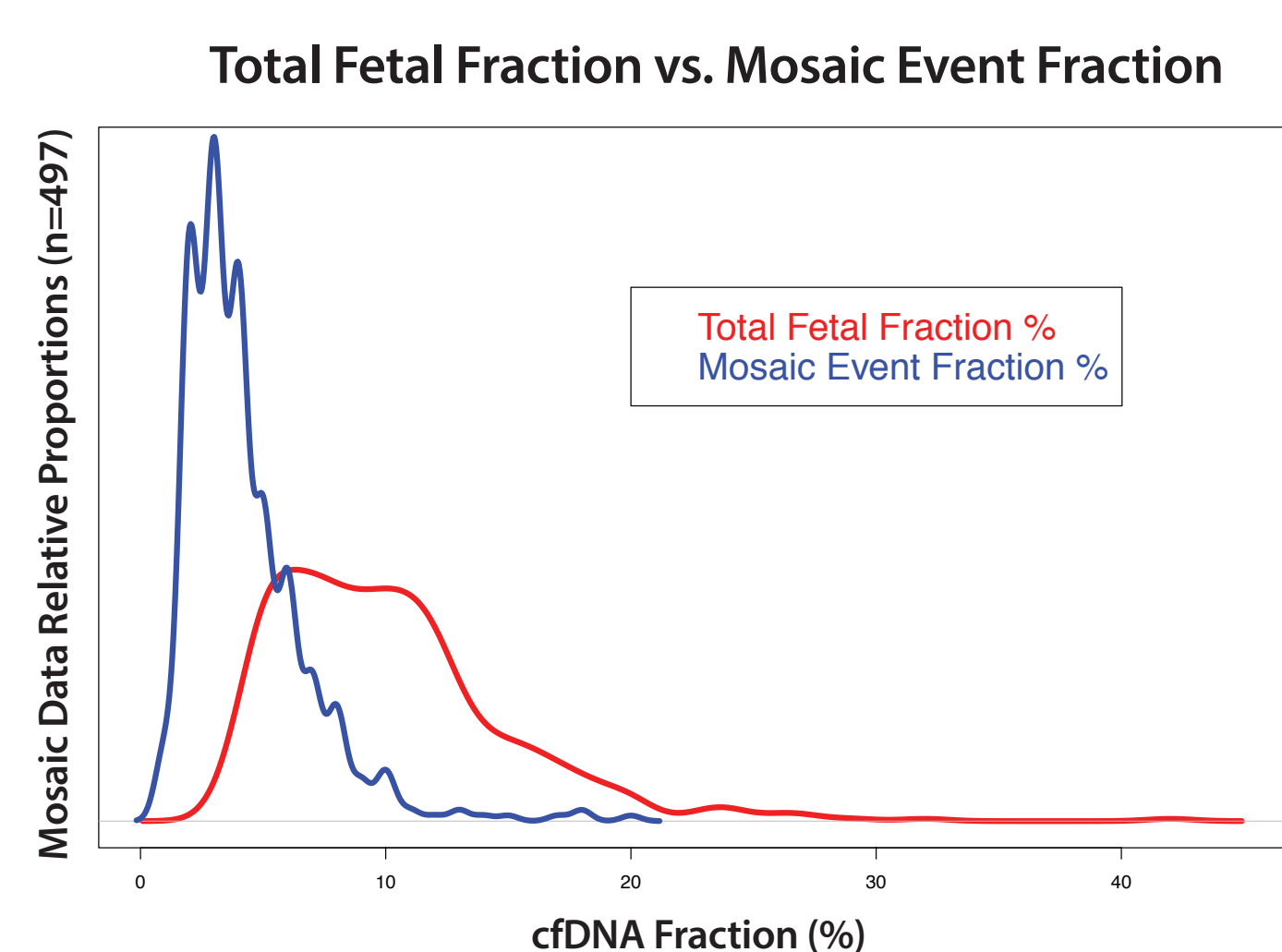
I. Introduction

The rapid evolution of prenatal cfDNA screening has expanded the once singular focus of trisomy 21 to now include genome-wide aneuploidy, microdeletions and large copy number variants (CNVs). This growth has brought with it a renewed appreciation for and awareness of placental mosaicism and the impact this biological phenomenon has on cfDNA data and interpretation. The resulting challenge lies in how best to integrate these advanced insights into clinical practice, while maximizing their clinical utility. Herein are summarized the laboratory methods and experience with mosaicism and correlation data with outcomes, with aim to construct a positive predictive model via logistic regression analysis.

III. Results

A total of 2,742 positive results were reported in the analyzed cohort, 497 (18%) of which yielded overt mosaic data (see **Image A**). Current laboratory protocol will generally include reporting results as 'mosaic' positives when the MR of the aneuploidy/CNV falls approximately between 0.2 and 0.7, in line with lower cytogenetic diagnostic thresholds for detecting mosaicism and our previous reported study that demonstrated a decline in relative positive predictive value (PPV) at MR values below 0.7.¹

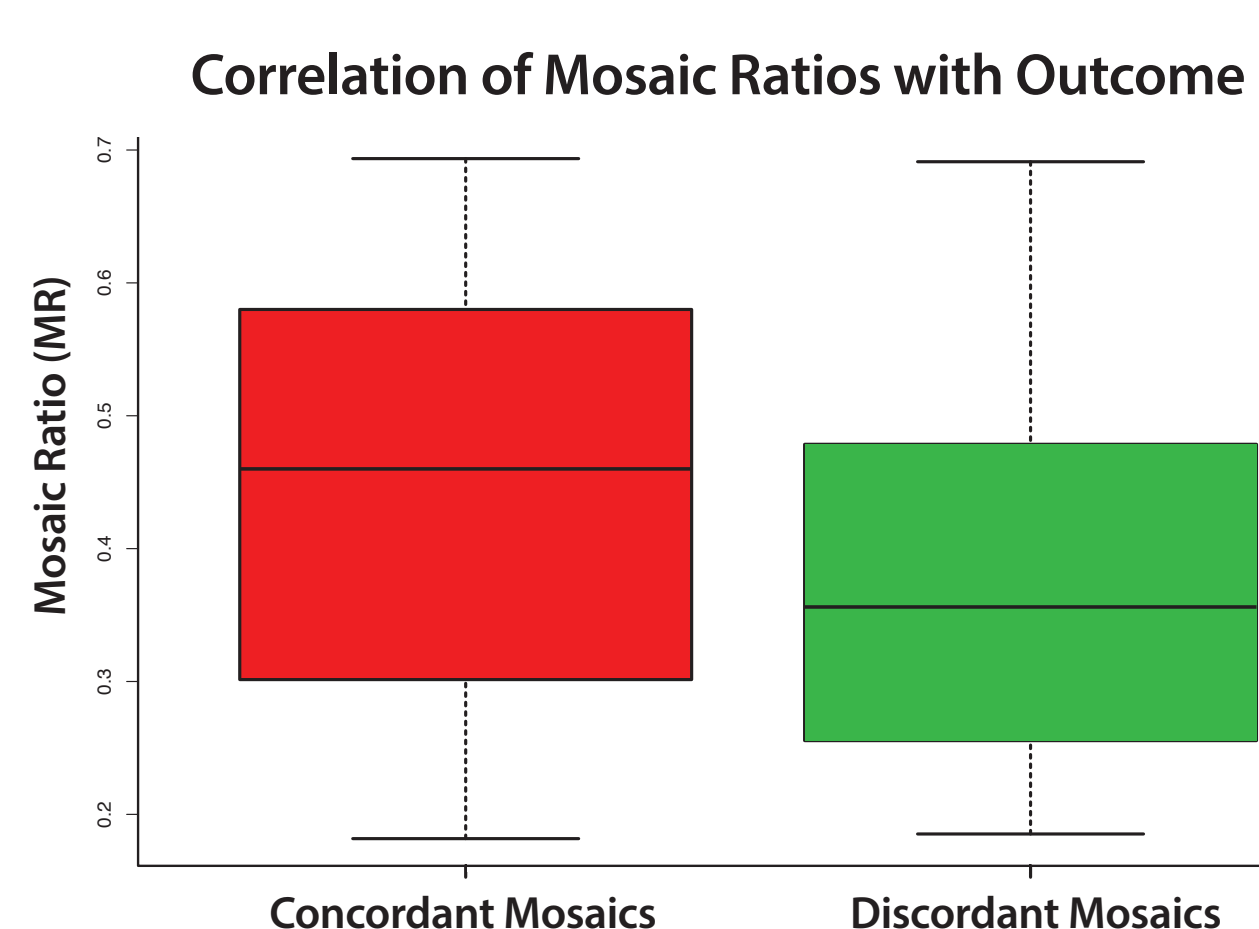
Image A. Mosaic cohort data 'fraction of abnormal event' compared to total cfDNA 'fetal fraction'.



The mosaic ratio mean among the mosaic cohort (n=497) was 0.43, with a median of 0.42, and standard deviation of 0.15. Fetal fraction mean for the cohort was 8.8%, with a median of 8.5%, and standard deviation of 5.7%. Incidentally, fetal fraction data was nearly identical between the mosaic (n=497) and non-mosaic (n=2,056) cohorts (p=0.902).

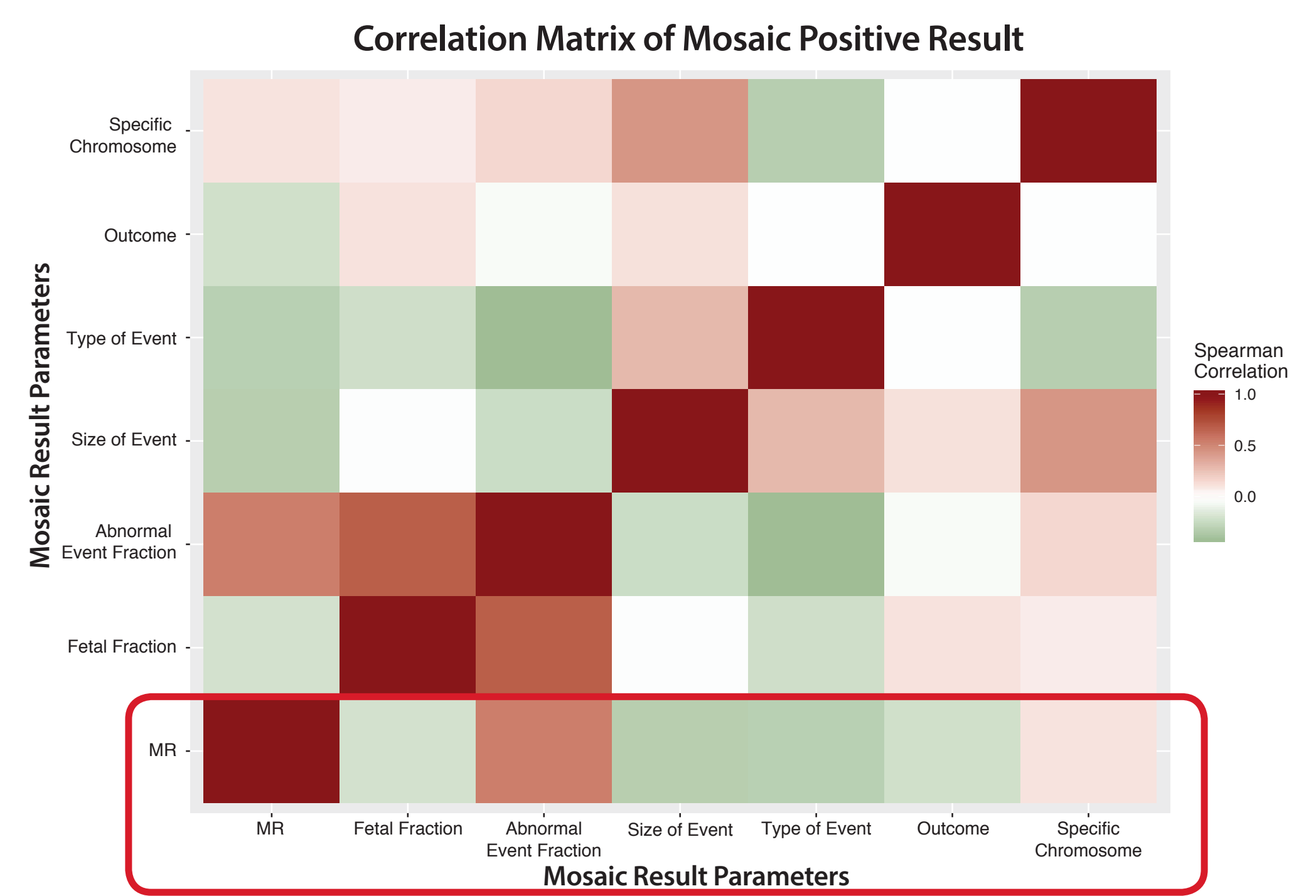
The overall discrepant positive rate (false positives) among the mosaic cohort was statistically significant, increased 5.6x higher compared to the non-mosaic cohort (28.3% vs. 5.0%, p<0.001) (see **Image B**). However, numerous noteworthy complications were reported among the discrepant mosaic cohort; including prior demise of a co-twin, subsequent intrauterine growth retardation (IUGR), intrauterine fetal demise (IUFD), pre-term delivery, and several cases of documented uniparental disomy (UPD) and/or confined placental mosaicism (CPM).

Image B. Correlation of mosaic ratios with outcome was found to be statistically significant, with concordant outcomes (true positives) having a mean MR of 0.45, median 0.46, and SD 0.15. In comparison, discordant outcomes' data (false positives) showed significantly lower MRs, with a mean of 0.37, median 0.36, and SD 0.14 (p<0.001).



Several multiple logistic regression models were tested. In addition to pregnancy outcome and mosaic ratio, additional variables were considered such as type of mosaic finding (e.g. trisomy vs. deletion), total cfDNA fetal fraction, abnormal mosaic event cfDNA fraction, size of abnormal event, and specific chromosome involved (see **Image C**).

Image C. Correlation matrix of the 7 key data parameters thought to potentially impact outcome concordance/discordance. Note relatively high correlation between 'Abnormal Event Fraction' and both 'MR' and 'Fetal Fraction'. Also of note, 'Size of event' is relatively highly correlated with 'Specific Chromosome' and 'Type of Event'. Negative correlation between 'MR' and 'Fetal Fraction', 'Outcome', 'Size' and 'Type' of event is also evident.



Ultimately it was determined that the addition of total cfDNA fetal fraction to outcome and mosaic ratio benefitted the model, based on AIC (Akaike's Information Criteria) statistic and overall p values. Indeed, mosaic ratio was found to be negatively correlated with discrepant outcome (at a statically significant level, p<0.001), while total cfDNA fetal fraction was slightly positively correlated with discrepant outcome (not individually statistically significant, p value=0.117). Undoubtedly other factors influence positive prediction (e.g. the specific chromosome involved) and will ultimately benefit the model, but limited size of the mosaic cohort precludes their inclusion at this time.

Multiple Logistic Regression Model Formula & Associated Calculations:

$$\text{logit} = \ln\left[\frac{p}{1-p}\right] = -\alpha - \beta_1(\text{MR}) + \beta_2(\text{cfDNA.FF})$$

$$p \text{ (aka 'probability for discordance')} = e^{\text{logit}} / (1 + e^{\text{logit}})$$

$$\text{PPV (aka 'probability for concordance')} = 1 - p$$

IV. Conclusion

Mosaicism is a common confounding factor in prenatal cfDNA screening. The quantification of mosaicism through a mosaic ratio (MR) has allowed for more accurate laboratory reporting and tailored interpretation. Correlating MRs with outcomes has shown MR to be inversely proportional to discordant diagnostic testing. While the discordance may be intuitive, discordant mosaic cfDNA results should not be mistaken as benign, as significant pregnancy complications are often reported.^{4,5}

Logistic regression modeling of outcomes with MR and cfDNA fetal fraction can assist with the probability prediction of concordant/discordant diagnostic testing. The proposed model is theoretically a useful additional tool for counseling patients about positive mosaic results. Future iterations will expand the model to be chromosome specific, in addition to including other key variables.

II. Methods

A retrospective cohort of nearly 56,000 samples submitted for genome-wide cfDNA prenatal screening during a three-year period (2015-2018) was analyzed, with special attention to mosaicism and available ad hoc clinical feedback on discordant results. As previously described^{1,2}, a mosaicism ratio (MR) of affected cfDNA to total fetal cfDNA was universally and routinely generated for all samples by dividing the fetal fraction estimated for the aberrant chromosome/segment over the fetal fraction estimated for all chromosomes. Resulting mosaic ratios were correlated with clinical outcome and total fetal fraction to develop a logistic regression model.

Bioinformatic statistical analysis was performed using RStudio software program v 1.1.456 - ©2009-2018.³ Statistical analyses included utilization of a 2 sample, 2 sided Z test for cohort comparison, Spearman correlation with pairwise deletion, and multiple logistic regression modeling of outcome probability (indicator variable) with mosaic ratio and fetal fraction data (continuous variables).

Mosaic trisomies constituted 66% of all mosaic results, followed by monosomies (18%), duplications (9.9%), and deletions (6%).

Images D & E. The frequency of mosaicism in our cohort appears highly influenced by the particular chromosome involved, as well as type of abnormality, and is in line with the broader placental literature findings.^{4,5} Monosomy X was the highest mosaic finding reported, followed by trisomy 7, 18, 13, and 21. The distribution of mosaic ratios also appears to be moderately influenced by the chromosome in question, consistent with the correlation data in Image C.

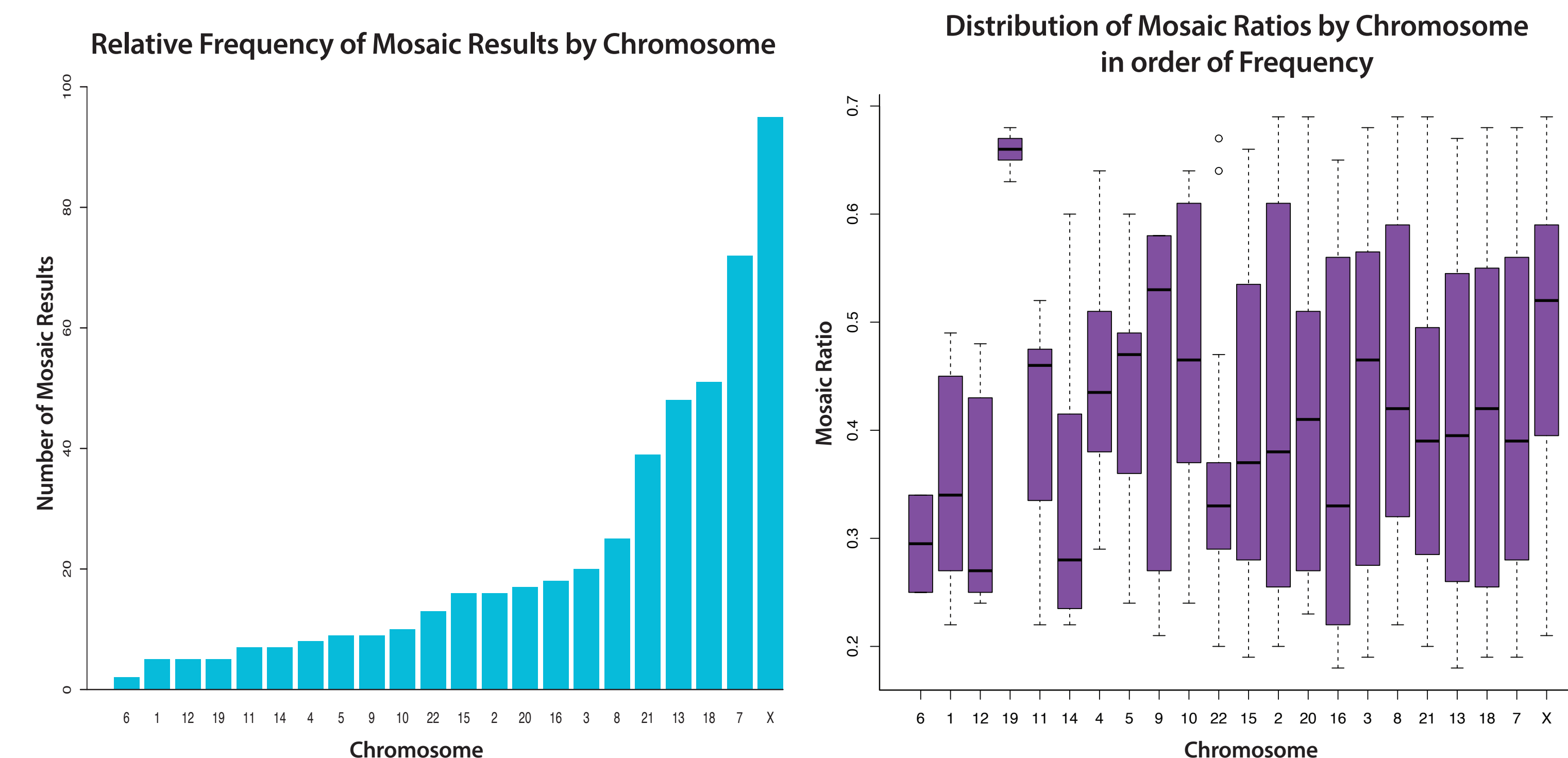
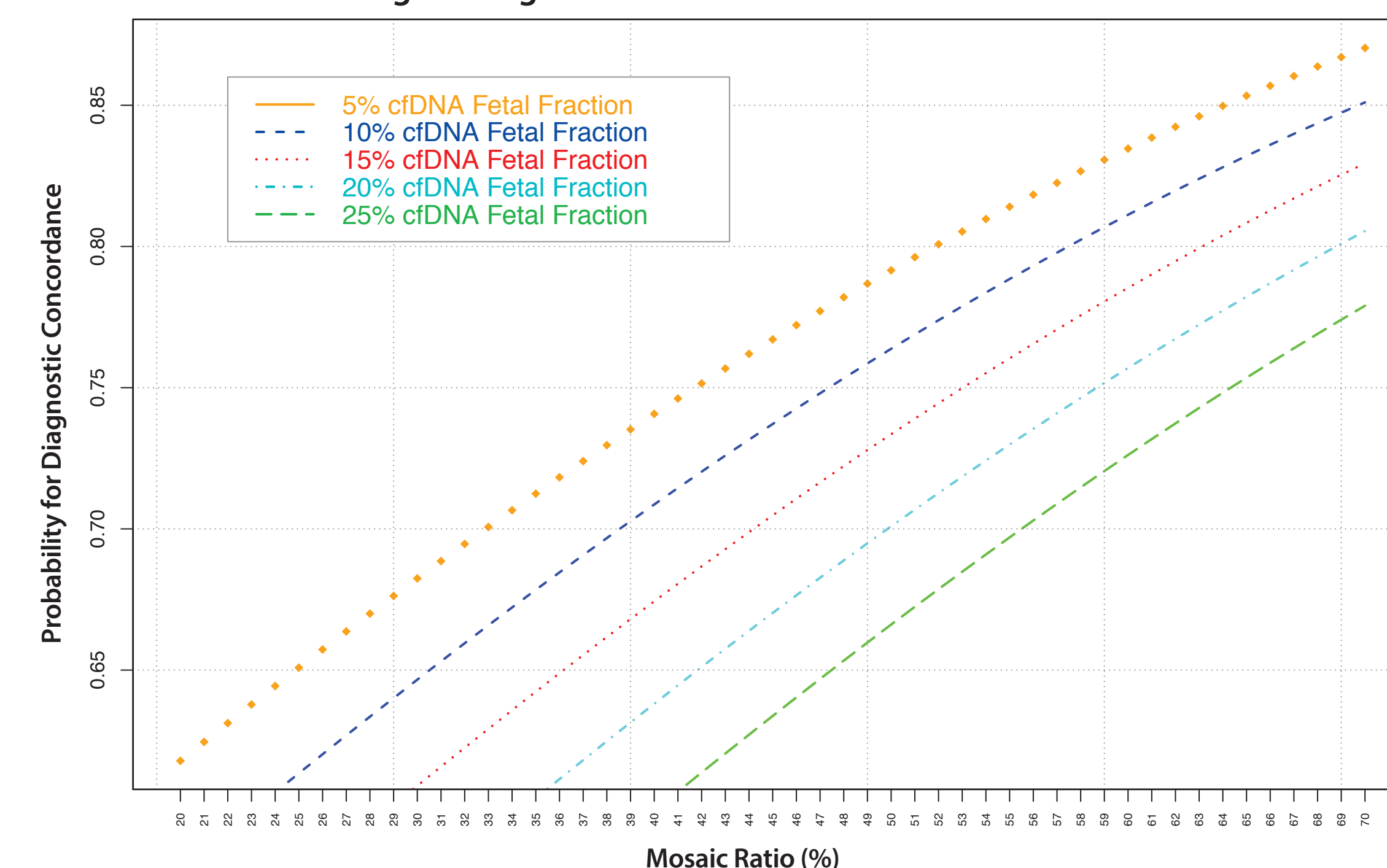


Image F. Depiction of the resulting probability values computed by the multiple logistic regression model across various cfDNA fetal fractions and the span of mosaic ratios used when reporting a positive mosaic result.

Positive Predictive Logistic Regression Model at various mosaic ratios & cfDNA fetal fractions



V. References

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