Application of cell-free fetal DNA concentration estimation by using fragment length difference in multiple gestations

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Background Cell-free fetal DNA concentration is a key



technical index, it refers to the proportion of cellfree fetal DNA in maternal plasma of pregnant women. It will result in false negative result in noninvasive prenatal test when fetal fraction is too low. Factors that affect fetal fraction including gestational age, maternal BMI and health condition of pregnant women. Therefore, research on cellfree fetal DNA concentration is the basis of improving accuracy and expanding adaptability of NIPT, especially in multiple gestations detection.

Method

We sampled 88 maternal plasma within which 57 were twin pregnancy and 31 were triplet

Figure 1 fetal fraction estimation by two methods



pregnancy. Conventional non-invasive pregnancy test was performed. Fetal fraction is estimated on the basis of fragment length difference between fetal DNA and maternal DNA in maternal plasma. Furthermore, we estimated fetal concentration by using chromosome Y and chromosome 21.

Result

Two fetal fraction estimated methods showed high consistency and correlation coefficient is R^2=0.81(Figure1). The concentration ratio of twins and single fetus is about 1.6, and that of triplets and single fetus is about 1.7 (Figure 2).Ratio (named k) of fetal fraction estimated from two methods was calculated (Table 1). Fetal gender was predicted by using k (Table 1). Furthermore, fetus number of chromosomal aberration was obtained, however which one is abnormal cannot be verified (Table 2).

Figure 2 relation of fetal fraction and gestational week

Table1 prediction of fetal sex by k-value in multifetal gestations

Ratio k	Twins	Triplets		
K=1	Two male	Three male		
K=2	One male and one female	-		
1 <k<2< th=""><th></th><th>two male and one female</th></k<2<>		two male and one female		
K>2		Two female and		

Conclusion

DNA fragment length method is feasible in fetal fraction estimation in multiple pregnancy. There is a proportional relation between cfDNA fraction and fetal number. Female fetal fraction can be estimated by this method. Moving on, larger sample size is needed to strengthen these results. one male **k-value on the** Two female Three female **Y-axis**

Table2 prediction of fetal sex and chromosome abnormality through estimating fetal fraction

Case ID	Fetus number	Fetal farction (Chr Y)	Fetal farction (fragment length)	Fetal farction (Chr 21)	Z- Chr21	Fetus gender	Fetus condition of Chr21
1	Twins	10%	10%	~0.03%	0.6	M/M	N/N
2	Twins	5%	10%	~0.03%	-0.6	M/F	N/N
3	Twins	10%	10%	10%	10.67	M/M	P/P
4	Twins	5%	10%	5.6%	5.8	M/F	P/N
5	Twins	~0	10%	5.6%	5.9	F/F	P/N
6	Twins	~0	10%	10.8%	10.96	F/F	P/P