Targeted cell-free DNA analysis with microarray quantitation for assessment of fetal sex and sex chromosome aneuploidy risk

The accuracy of targeted cell-free DNA (cfDNA) testing with DANSR™ and FORTE™ for trisomies 21, 18 and 13 has been well demonstrated and is consistent across next generation sequencing and microarray quantitation methods[1]. Targeted cfDNA analysis for fetal sex chromosome aneuploidy (SCA) has also been validated and shown to have high specificity in prospective studies[2,3]. This study expands upon the available published data by investigating the performance of targeted cfDNA analysis of the X and Y chromosomes using microarray quantitation for assessment of SCA probability in singleton pregnancy and fetal sex in twin and singleton pregnancies.

Samples of banked maternal plasma from 791 singleton and 51 twin pregnancies were obtained as part of ongoing multicenter clinical studies (NCT02201862 and NCT01451671) and from a sample bank at King's College London, UK. Single cell-free Roche, Streck BCT-DNA or EDTA collection tubes were available for each sample. Collection and processing differed from commercial protocols only in that all samples were frozen prior to analysis and available specimen volumes were lower than standardly used. Patient consent and fetal karyotype information was obtained for all samples. The cohort included 15 singleton pregnancies with sex chromosome aneuploidy. Targeted cfDNA analysis with microarray quantitation was performed, as previously described, using a blinded protocol[4].

Y-chromosome specific DANSR assays were used to evaluate fetal sex in twin and singleton pregnancies. Results were reported as male or female, depending on concluded presence or absence of Y-chromosome fragments. In twin pregnancies, a male result indicates the presence of at least one male fetus.

Fetal SCA analysis was performed on samples from singleton pregnancies using X- and Y-specific DANSR assays followed by FORTE analysis adapted for this purpose[2,4,5]. A probability cut-off of 1 in 100 for non-disomic genotypes was used for calculation of sensitivity and specificity.

Gestational age and fetal fraction averaged 16.7 weeks and 13.4%, respectively. Thirty-nine singleton and 12 twin pregnancy samples had insufficient fetal fraction or failed to pass quality control thresholds resulting in 752 and 39 samples undergoing testing for fetal sex in singleton and twin pregnancies, respectively. Fetal sex results were yielded in 748/752 singleton and 39/39 twin samples. Of these, predicted fetal sex was consistent with karyotypic sex in 786/787 cases (99.9% concordance) (Table 1). All twin fetal sex cfDNA results accurately reflected either the presence of two female fetuses ($n = 18$) or at least one male fetus ($n = 21$).

For SCA assessment, 742 samples were eligible. All 15 cases of SCAs were correctly identified (100% sensitivity; 95% CI, 79.6–100%) (Table 1). Out of 727 disomic (XX or XY) pregnancies, 725 were correctly classified as low-risk for SCA (99.7% specificity; 95% CI, 99.0–99.9%) (Table 1).

In summary, targeted cfDNA analysis performed with high accuracy for fetal sex assessment in twins and singletons, and correctly identified all SCAs with high specificity. A limitation of using these banked samples is that the positive predictive value observed in this enriched cohort would not be translatable to a routine prenatal screening population. In addition, the number of samples passing quality thresholds may be lower than standard due to irregular sample volumes. However, this study provides a valuable supplement to the currently available data supporting the use of targeted cfDNA analysis for fetal sex and SCA assessment and substantiates previous conclusions that the performance of this methodology is robust across quantitation platforms.

Table 1 Performance of cell-free DNA (cfDNA) testing for fetal sex in singleton and twin pregnancy and for sex chromosome aneuploidy in singleton pregnancy in current study and previous publications

			Sex chromosome aneuploidy						
	*Fetal sex accuracy**		*45,X†*		*47,XXX†*		*47,XXY†*		*Disomy accuracy**
Study	*Singleton*	*Twin*	*DR*	*FPR*	*DR*	*FPR*	*DR*	*FPR*	
Current	747/748 (99.9 (99.3–100))	39/39 (100 (91.0–100))	13/13 (100 (77.2–100))	1/742 (0.1 (0–0.8))	1/1	1/742 (0.1 (0–0.8))	1/1	0/742 (0 (0–0.5))	725/727 (99.7 (99.0–99.9))
Hooks[2]	414/414 (100 (99.1–100))	—	26/27 (96.3 (81.7–99.3))	2/380 (0.5 (0.2–1.9))	1/1	2/380 (0.5 (0.2–1.9))	6/6	0/380 (0 (0–1))	378/380 (99.5 (98.1–99.9))
Nicolaides[3]	109/110 (99.1 (95–99.8))	—	43/47 (91.5 (80–96.6))	0/172 (0 (0–2.2))	5/5	1/172 (0.6 (0.1–3.2))	1/1	0/172 (0 (0–2.2))	115/116 (99.1 (95.3–99.9))

Only first author of each study is given. Data are presented as *n*/N (% (95% CI)). *Accuracy defined as concordant cfDNA and karyotype test results. †Sensitivities for individual sex chromosome aneuploidies cannot be concluded due to small number of affected pregnancies. DR, detection rate; FPR, false-positive rate.

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Disclosures

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