Genome-wide cfDNA screening: AMES laboratory experience with 10,500 pregnant women

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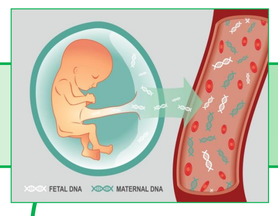


Introduction and Aims

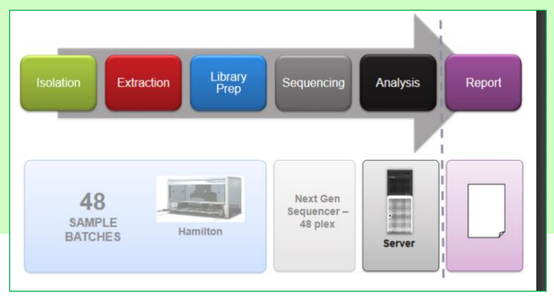
The “traditional” cfDNA screening tests are tipically limited to a selected subset of chromosomal abnormalities, including trisomies 21, 18 and 13, as well as sex chromosome aneuploidies. Whole-genome sequencing (WGS) of maternal plasma cell-free DNA (cfDNA) can potentially evaluate all 24 chromosomes to identify abnormalities of the placenta, fetus, or pregnant woman. We hypothesized that by systematically analyzing WGS data from all chromosomes, we could identify rare autosomal trisomies (RATs) to improve understanding of feto-placental biology. We also aimed to compare the performance of the two tests in a this large population of pregnant women, in order to assess the clinical utility of the genome-wide screening.

Materials and methods

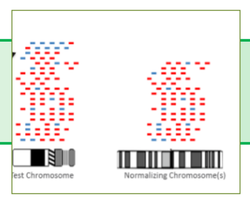
We analysed 10500 pregnant women for genome-wide cfDNA screening.

Sequencing libraries were prepared using VeriSeq NIPT 48plex kit (Illumina, San Diego, CA, USA)

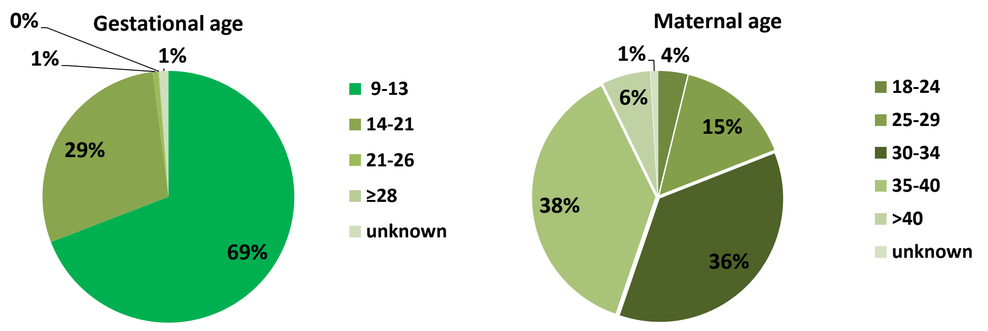
Sequencing data were analyzed using an algorithm optimized to identify aneuploidies and subchromosomal aberrations[1].



1. Bayindir B, et al.
Eur J HumGenet 2015;23:1286–93



Results

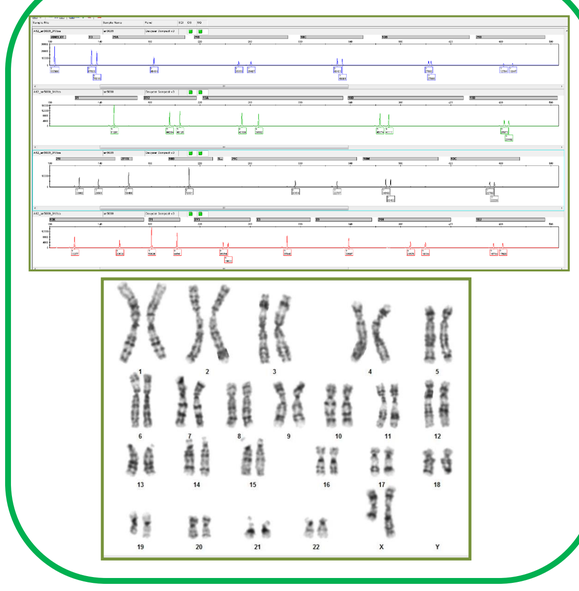


The mean age of pregnant women was 34.5±3.7 years (range,18-47) and fetal fraction was 9.5±3.7. Screen positive test results were reported in 164 cases, leading to a screen positive rate of approximately 1.6%, and confirmed by karyotyping or array-CGH following invasive prenatal diagnosis in 155 (1.5%) cases. Trisomies involving chromosomes 21 (N=65), 18 (N=22) and 13 (N=11) are most frequently reported (1.2%). A total of 30 samples were reported to screen positive for aneuploidies of autosomes other than chr 21, 18 and 13. Most often affected were chr16 (12 cases), chr7 (7 cases), chr22 (6), chr3 (5). 33 aneuploidies involved sexual chromosomes (22 X0 and 11XXY). Subchromosomal events were reported on all autosomes with the exception of chr19 and 17. Also an unbalanced translocation event was found t(3;5)(q24;p15) and X deletion (46,XX,del(X) (q?). Overall, clinical sensitivity and specificity were 100% and 99.78%, respectively.

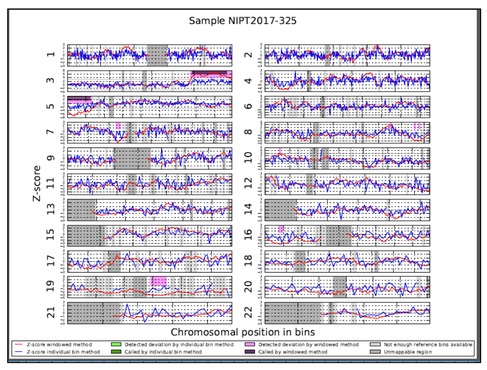
	Table 1*: Performance of the genome-wide cfDNA screening approach (N=10500)			
	Trisomy 21	Trisomy 13	Trisomy 18	Sex chromosome Aneuploidies
Sensitivity	100% (65/65)	100% (11/11)	100% (22/22)	100%(33/33)
95% CI	(99.8%,100%)	(79.8%,97.2%)	(87.6%, 100.0%)	(77%,100%)
Specificity	100% (10435/10435)	>99.9% (10488/10489)	>99.9% (10476/10478)	>99% (10457/10467)
95% CI	(99.8%, 100%)	(99.7%,100%)	(99.7%, 100%)	(99.9%,100%)

Case1

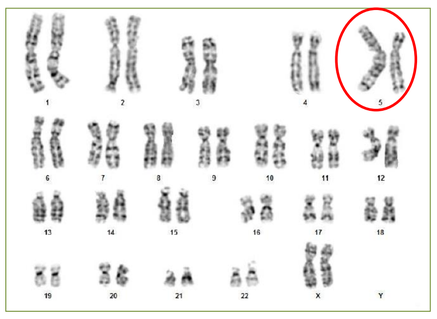
In **Case 1**, NIPT revealed a X0 female which was not supported by QF-PCR of amniocyte DNA, while final karyotype showing partial deletion of long arm of X chromosome. In **Case 2**, a deletion in the proximal long arm of chromosome 3 of paternal origin (46,XX,der(5)pat) was suspected, as showed with NIPT, and confirmed by karyotype of paternal white cell DNA.

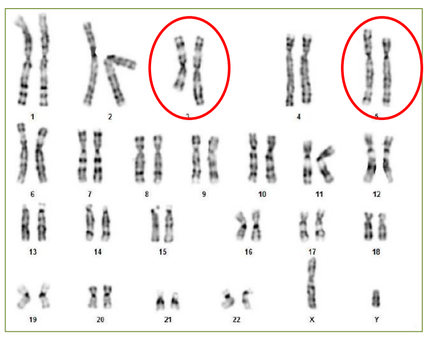


Case2



46,XX,der(5)pat





46,XY,t(3;5)(q24;p15)

Conclusions

This report provides our clinical experience with genome-wide cfDNA analysis for prenatal diagnosis. Genome-wide screening allowed detection of 30 (7.4%) potentially viable clinically relevant chromosomal abnormalities, which would have remained overlooked if only conventional NIPT had been performed.