Noninvasive detection of chromosomal CNV and single gene disease by massively parallel sequencing of cfDNA

Ya Gao1, TK Lau2, Chenming Xu3, Zhengfeng Xu4, Zhiying Gao2, Shenpei Chen5, Xuchao Li1, Chunlei Zhang1, Xiaoming Wei1, Fang Chen1, Wei Wang1

1BGI-Shenzhen, Shenzhen, China, 2Fetal Medicine Center, Hong Kong, Hong Kong, 3Zhejiang University, Hangzhou, China, 4Nanjing Maternity and Child Healthcare Hospital, Nanjing, China, 5Chinese PLA General Hospital, Beijing, China

OBJECTIVES: Cell-free DNA based non-invasive prenatal testing (NIPT) has been shown with close-to-diagnosis sensitivity and specificity in detecting fetal T21, T18, and T13. Future development of this technology focuses on detection of other chromosomal aneuploidies, and more importantly chromosomal copy number variants (CNVs) and single-gene mutations. Validation studies were carried out to demonstrate the feasibility of detecting CNVs and single-gene disease by exploiting the cell-free DNA testing strategy and existing NIPT platforms.

METHOD: For testing CNVs, 35 plasma samples with known fetal CNVs (>10 Mb in size) were recruited, together with 1077 plasma samples with normal fetal karyotypes as the control. The samples were blindly tested using the routine NIPT testing pipelines adopted at the clinical laboratories of BGI, and analyzed with the proprietary FCAPS protocol. Results were compared to the karyotyping results to calculate the sensitivity and specificity of the NIPT test. For testing single-gene disease, we developed a unique haplotype-assisted approach which was validated in a Maple Syrup Urine Disease (MSUD) case. In a trio family (father, mother in early pregnancy, and proband), targeted sequencing (180X) of the known genes causing MSUD was used to identify the disease causative mutations in the proband child and parents.

RESULTS: For CNV detection, with on average 4–5 M reads per sample and the existing NIPT platforms, we were able to use the FCAPS analytic protocol to identify 32 positive samples in total of 35, and generated 6 false positive cases, leading to overall sensitivity and specificity of 91.4% and 99.4% respectively. False positive rate and false negative rate of the test were 0.56% and 0.28% respectively. We also showed that with an increased sequencing depth, the detection power of the test can be further improved. In the MUSD case, deep targeted sequencing in the trio family identified that the proband child carries a missense mutation and a large deletion on BCKDHA gene, which were inherited from the mother and father respectively. With the SNP markers of the proband and parents, parental haplotype of chromosome 19 in 6148 loci were recovered. After fetal haplotype construction, the same alleles as that of the proband in the causative gene BCKDHA were inherited to the fetus, indicating that the fetus is a MSUD patient. This result was later confirmed by invasive diagnosis, and led to a well-informed decision after post-test counselling.

CONCLUSIONS: We demonstrate the successful application of NIPT in detection of CNVs larger than 10 Mb without changing much to the current pipelines of aneuploidy detection. It has the potential to be integrated into the current NIPT test and offer extra benefits to the clinical service. The haplotype-assisted approach we developed requires minimum genetic information to accurately predict fetal condition. It has the clinical potential for non-invasive prenatal diagnosis of single gene disease for family with a proband child and seeks a second healthy child.

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mutant to normal allele calculated. Using simple
Mendelian inheritance rules and assuming that the
background maternal alleles are equally represented
in the plasma, the fetal genotypes could be deduced.
RESULTS: Four WD family pedigrees with an
affected proband consented to the study with the
aim of identifying the pathogenic mutations and
offering prenatal diagnosis for a second conceived
child. Sanger and whole exome sequencing mapped
the causative ATP mutations in the parental, mater-
nal and proband DNA samples, and in each family,
both a known and novel mutation was identified. All
four couples conceived a second child and invasive
prenatal diagnosis identified two heterozygous car-
rier, one normal and one affected fetus. Retrospec-
tively, we analysed the maternal plasma samples
from each pregnancy that has been collected and
frozen following amniocentesis. In preliminary val-
idity experiments, we first showed that cSMART
could quantitate the correct percentage of mutant
alleles in plasma samples seeded with different
amounts of known SNPs to mimic both paternal
and maternal inheritance of the fetal alleles. We then
applied our validated NIPT assay to the pregnancy
plasma samples and showed that the fetal genotypes
assigned by Sanger sequencing of amniocytes were
concordant with the fetal genotypes assigned by
cSMART. CONCLUSIONS: A reliable and accu-
rate assay called cSMART was developed that
correctly diagnosed the fetal genotypes in maternal
plasma samples from four pregnancies at risk for
WD. This method has the potential for NIPT of
other monogenic disorders since it only requires the
knowledge of the pathogenic mutations and/or
linked SNPs.

LB-5
The impact of aspirin on the prevalence of early onset
pre eclampsia after first trimester screening
Felicity Park1, Kate Russo1, Marilena Pellosi2, Rachel Puddephat2, Mary Walter2, Constance
Leung1, Rahmah Said1, Hasan Rawashdeh1, Jon Hyett1
1University of Sydney, Sydney, Australia,
2Royal Prince Alfred Hospital, Sydney, Australia

OBJECTIVES: Several studies have shown that
demographic, biophysical and biochemical param-
ters can be combined to screen for early onset pre-
 eclampsia (ePET) at 11–13 + 6 weeks’ gestation.
Meta-analyses support the use of Aspirin (<16 weeks) as a therapeutic intervention to reduce the
prevalence of ePET and improve neonatal morbidity. We aimed to demonstrate the value of
first trimester prediction and intervention for
ePET. METHOD: This is a retrospective analysis
of two consecutive cohorts screened for ePET. The
first cohort, screened between 16 April 2010 and 9
March 2012, were observed and used to validate the
FMF ePET algorithm. Women were screened using
demographic history, mean arterial pressure, uterine
artery Doppler and PaPP-A. High-risk women (>2%
ePET risk) in the second cohort (1 April 2012 to 5
June 2103) were advised to take Aspirin (150 mg,
nocte) to 34 weeks’ gestation. Pregnancy outcomes
were collated from the State mandated midwifery
dataset. Case notes of women delivered <34 weeks’
gestation were reviewed to ensure accuracy of
registry information. RESULTS: 3066 women were
screened for ePET in the observational cohort; 12
(0.4%) of these women required delivery <34 weeks.
2515 women were screened in the therapeutic cohort;
250 (9.9%) women were high risk and advised to
take Aspirin; 1 (0.04%) developed ePET (chi-
squared: P = 0.01). There were no cases of ePET
reported in women who had a low risk in this second
cohort. CONCLUSIONS: The FMF algorithm pre-
dicting risk of ePET appears to be effective in an
Australian population. Aspirin effects a significant,
ten-fold reduction in the prevalence of ePET.