Advanced Technology Development in Cell Free DNA in Maternal Blood

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Istanbul, Turkey
November 30th, 2015

Turkish Gynaecologist Congress 2015
Disclosure

Fang Chen is an employee of BGI-Shenzhen and the Director of Reproductive health and fetal medicine in Science and Technology for seven years.

BGI-Shenzhen is non-profit research institute in China and focus on science and research, both in fundamental mechanism and clinical practice.

BGI genomics co., LTD. offers genetic testing service for clinical application and sequencing & analysis service for scientific research.
BGI – One of The largest Genomics Institution

>5,000 employees  >200 NGS platforms  Global existence
Core Facility for Genomics Medicine

DNA Seq, small RNA-Seq, ChIP-Seq, Transcriptome, DGE, DNA Methylation, Target Region Sequencing, Meta-Genome Seq

Bioinformatics, National Gene Bank, Big Omics data

The Power of Informatics
Get the Most Out of Your Data

Proteomics, Metabolomics, Lipodomics, Biomarker Discovery, ID, Verification, Structure, Quantification, Target & Non-Target Analysis
The past six years (2008-2014) has witnessed the quick development of sequencing technology as well as the fast transfer of this new technology from basic science research to practical clinical application (from dream to reality).
Next Generation Sequencing (NGS)

- The breakthrough of the fast transfer were highlighted in two aspects during 2008-2014
  - Whole exome sequencing for identifying novel gene mutations for single gene disorders & Target capture sequencing for interpretation of clinical cases in known genes
  - Maternal plasma DNA sequencing for noninvasive prenatal detection of Down’s Syndromes

Several new cross discipline between biology and computer science appeared, such as genetics, genomics, population genetics, comparable genomics, bioinformatics, biological statistics, biomathematics, et al.
Single gene disorders and NGS

From discovering novel gene mutations to clinical testing of causative gene mutations

• The first publication to describe whole Exome sequencing for identifying novel gene mutations for diseases in 2008

• Exome:
  – Total exons in a genome
  – ~1-2% of the genome
  – Coding region
  – 75-85% disease causative mutations
  – Efficient method to discover mutations
  – Straightforward molecular evidence
Single gene disorders and NGS

clinical testing of causative gene mutations

• target capture sequencing

• Target:
  – Selected number of gene with clear clinical significance
  – Target sequence capture
  – Easy result explanation
  – Cost effective
NIPT and NGS

- NIPT: noninvasive prenatal testing (*screen the high-risk subgroup from general population*)
- NIPD: Noninvasive prenatal diagnosis testing (*diagnosis in a noninvasive way*)
- NIFTY: Non-invasive fetal trisomy test

- NIPT refers to a wide scope of all prenatal cares in a noninvasive or minimal invasive way. Yet currently, NIPT is mostly related to the cell free DNA-based testing of genetic diseases, especially chromosomal aneuploidies.
Milestone for NIPT

1950: Discovery of trophoblasts
1979: FACS
1990: Successful enrichment of fetal cell by FACS
2005: Failure

1970: Discovery of fetal lymphocytes
1980: Successful determination of fetal aneuploidy
1990: Successful enrichment of fetal nucleated red cells
2000: NGS-based NIPT technology
2010: cFDA approval
2011: NIPT Clinical service
2014: CE marked
2015: Generally accepted

1997: Scientific Discovery
2008: breakthrough
2011: NIPT era
2014: Generally accepted
2015: CE
Characteristics of cell free DNA in maternal plasma

➢ Discovery
- Chromosome Y specific sequence observed in the plasma of pregnant women with male fetus
- Naturally fragmented 150-200bp DNA
- Detectable as early as 5th gestational weeks

➢ Origination
- Derived from placental trophocytes
- Disappears soon after childbirth (Placental expulsion)

➢ Fraction
- cffDNA presents 5-30% of cell free total DNA in maternal plasma and diverse greatly in individuals
- Increase with the gestation age, aneuploidy pregnancies and decreases with BMI
### Sequencing strategies

#### Whole genome sequencing
- Non-polymorphism unique region in the human genome (>95%)
- Aneuploidies in 24 chromosomes and smaller CNVs analysis by reads density in automated bioinformatics analysis
- Can discriminate standard T21, partial T21 and mosaic T21
- Fetal fraction can be estimated by chromosomal Y specific or trisomy chromosomal specific sequence

#### Target region sequencing
- Polymorphism region in the human genome (1/1000-1/100)
- Aneuploidies at selected chromosomes (21/18/13/X/Y) and target regions by reads density or SNP ratio in automated bioinformatics analysis
- Fetal fraction can be estimated by chromosomal Y specific or father-inherited SNP ratio information

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**Note:** Currently, all the IVD kit approved by cFDA and CE are based on Whole genome sequencing strategy
Fetal fraction (important, but not unique)

- Cell free fetal DNA comes from placenta other than fetal cell or tissues, and detectable till the placenta structure functions.

- When the placenta structure stay stable, indispensable exchange in both sides (oxygen, small molecular, et al); placenta cells drop off and into maternal blood circulation, forming 166-bp apoptotic body.

- As the gestational weeks increases, the exchange and apoptosis rate increases with the pregnancy; a fundamental need to support a live fetus.

- There should be minimal and maximal fraction of cell free fetal DNA in maternal blood circulation during 280 days.

Fetal fraction (important, but not unique)

<table>
<thead>
<tr>
<th>Simulated plasma#</th>
<th>male</th>
<th>female</th>
<th>test positive</th>
<th>test negative</th>
<th>unclassified*</th>
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<tr>
<td>T21</td>
<td>10.00%</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>0</td>
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<tr>
<td></td>
<td>3.50%</td>
<td>15</td>
<td>15</td>
<td>18</td>
<td>0</td>
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<tr>
<td></td>
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<td>15</td>
<td>15</td>
<td>4</td>
<td>15</td>
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<tr>
<td></td>
<td>10.00%</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>T18</td>
<td>5.00%</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>0</td>
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<td>2.50%</td>
<td>15</td>
<td>15</td>
<td>3</td>
<td>17</td>
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<tr>
<td></td>
<td>10.00%</td>
<td>15</td>
<td>15</td>
<td>30</td>
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<tr>
<td>T13</td>
<td>5.00%</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>0</td>
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<tr>
<td></td>
<td>3.50%</td>
<td>15</td>
<td>15</td>
<td>13</td>
<td>0</td>
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<tr>
<td></td>
<td>2.50%</td>
<td>15</td>
<td>15</td>
<td>6</td>
<td>13</td>
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<td>Negative control</td>
<td>46,</td>
<td>10.00%</td>
<td>15</td>
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<td></td>
<td>XN</td>
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<tr>
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<td>15</td>
<td>15</td>
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<tr>
<td></td>
<td>2.50%</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>30</td>
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</table>

-Unpublished data from BGI

# simulated plasma served as part of National reference materials for NIPT for cFDA approval

* unclassified cases needs resampling
Fetal fraction

(1) Increases with gestational weeks.
   - 1% increasing between 10 to 22w
   - increased more rapidly after 23w (~ 1% /week)

(2) Negatively correlates with maternal BMI
    (by partial correlation analysis)

(3) T21 pregnancies > euploid control*
    T18 pregnancies < euploid control*
    (* controls with same gestational weeks and BMI)
Opinions of NIPT from Organizations

Summary:
- NIPT can be used as an option for aneuploidy assessment in pregnancy.
- NIPT can effectively detect fetal trisomy 21, trisomy 18 and trisomy 13, but has not yet been shown to be efficacious in detecting other chromosomal abnormalities or single-gene disorders.
- Currently there are not enough studies to support NIPT as a routine, first-tier aneuploidy screening test in low-risk populations.
- Pre- and post-NIPT genetic counseling is necessary for all women considering NIPT.
NIPT story in BGI

2006-07  NGS established at BGI
2007-09  NGS-based NIPT technology development
2009-12  Multi-centered validation study with local hospitals
2011-    NIPT into clinical practice (for T21, T18 and T13)
2012    Expert opinion published in China
2013    NIPT into clinical practice (for 1p36 del, 5p del and 2q33.1 del)
2014    cFDA approval for NIPT (for T21, T18 and T13)
2015    CE approval for NIPT (processing)
2016    BGISEQ-500 for NIFTY

NIPT Beyond Fetal Aneuploidy

Fetal Microdeletion/microduplication Syndrome

- Sliding window to analyze the genome sequence
- Can detect > 5Mb deletion/duplication
- Requires more sequencing data compared to aneuploidy testing
- Not provided as a routine test till fully validated
- Smaller CNV as second findings for research use on

Table 2. MPS Results on Maternal Plasma Samples that Are Concordant with the Clinically Reported Karyotype

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Affected Chr</th>
<th>Gain/Loss</th>
<th>Start Bin</th>
<th>End Bin</th>
<th>Size (Mb)</th>
<th>Chromosome Region</th>
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<td>BE3096</td>
<td>6</td>
<td>gain</td>
<td>64</td>
<td>102</td>
<td>38</td>
<td>6q12–6q16.3</td>
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<td>7</td>
<td>gain</td>
<td>98.1</td>
<td>98.3</td>
<td>0.3</td>
<td>7q22.1</td>
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<td>19</td>
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<td>BC2659</td>
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<td>gain</td>
<td>158</td>
<td>198</td>
<td>40</td>
<td>3q25.32–3q29</td>
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<tr>
<td>X</td>
<td>loss</td>
<td>1</td>
<td>10</td>
<td>9</td>
<td>Xp22.33–Xp22.31</td>
<td></td>
</tr>
</tbody>
</table>

David et al., 2011; Taylor et al., 2012; Anupama et al., 2013; Chen et al., 2013
NIPT Beyond Fetal Aneuploidy

Monogenetic diseases

- Uses target-enrichment capture sequencing
- More than 20 single gene disorders with high prevalence in newborns, e.g. Thalassemia, caused by different mutation types (CNV, SNV, Indel)
- Information of proband is required
- Not be provided as a routine test
- Over 200 clinical samples in 2013-2015 with TAT of three weeks
Since 2011, **32** papers have been published on SCI journals:

NGS methods, bioinformatics, multicenter clinical research, twin, CNV, additional findings, fetal genome, etc.

Till October 2015, over **740,000** cases done.
Empowering Genomics-Based Healthcare

**Middle-age**
- Old-age
  - Testing for personalized cancer treatment
  - Hereditary cancer screening
  - Genetic testing for infectious disease (HPV, HBV, HCV)

**Newborn**
- Children
  - Youth
    - AngelCare™ (genetic and metabolic disease screening)
    - Whole exome sequencing
    - Stem cell storage and HLA high resolution genotyping

**Prenatal**
- Pre-conception
- Pre-implantation
  - Non-invasive Fetal Trisomy (NIFTY) Test
  - Non-invasive prenatal testing for monogenic disease

**Pre-conception**
- Genetic disease screening
- Pre-implantation screening (PGS)
- Pre-implantation diagnosis (PGD)
- Genetic testing for recurrent miscarriage and malformation
Meet the challenges of NIPT in clinical practice

- **Science and Technology**
  - Whole genome sequencing/ Target sequencing
  - Simple personalized array design
  - Faster, cheaper and smaller sequencer

- **Education**
  - Genome Biology/ Genomic Medicine/ Genetic Disorders Screening/ Genetic Counseling/ Molecular Biology/ Bioinformatics/ Medical genetics

- **Guidelines and Standardization**
  - Lab: MOH/ ISO15189/ CLIA/ Reagent/ Sequencer: cFDA/ FDA/ CE/ KFDA
  - Manipulator: qualified?

- **Social Obligation and Humanity**
  - Healthcare system/ Universal education
  - One-child policy in China/ Sex selection
  - Drugs and treatment
NIFTY on BGISEQ-500

- Turnaround time with 24 hours
- Fully automated library preparation/User friendly operation-Pre-optimized kit/Automated data analysis
- High/low throughput for different clinical use (16-192 NIFTY samples)
- Already start to apply for cFDA/CE/FDA approval
- Planning to give the service in 2016
Research

Whole genome sequencing
Long Fragment Reads sequencing
Whole exome sequencing/Target panel
Plant/Animal de novo/resequencing/Tag-Seq
Fungal/Bacteria genome de novo
Metagenomics
16S/18S amplicon sequencing
RNA-seq(Transcriptome/Quantification)
Single cell RNA-seq
Small RNA/ncRNA-seq
Epigenetics( WGBA/RRBS/MeDIP-seq/ChIP-seq)

Clinical

NIFTY
EmbryoSeq
ChromoSeq
Carrier test
Nova Newborn SCREENING/HearingCare
Monogenic Diseases testing
Osmart™ BRCA genetic test
Osmart™
Lung/Breast/Colorectal/Gastric/Liver/Blood and Lymph cancer personalized therapy genetic test
Pathogens detection service
HPV/HBV/HCV genotyping
HBV drug resistance

Incoming 24-hour NIFTY/PGS/Chromo test on BGISEQ-500

www.seq500.com
Acknowledgment

We thank the colleagues in BGI and our collaborators all over the world!

We thank our collaborators in Turkey! Genoks!
Thank You!

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