SNP Microarray Genetic Analyses to Determine 23-24 Chromosome Ploidy, Structural Chromosome Aberrations and Genome-Wide Scans to Identify Disease Risks from a Single Embryonic Cell

WG Kearns

Shady Grove Center for Preimplantation Genetics

Shady Grove Fertility Reproductive Science Center

Rockville, MD   USA
Indications for Genetic Assessment of Embryos

- Aneuploidy Screening (PGS)
- Structural Chromosome Aberrations (PGD)
- Single Gene Disorders (PGD)
- Mitochondrial Disorders (PGD)
Cell Types - Biopsy

- Polar Bodies
  - Meiosis I errors
  - Meiosis II errors
  - X-linked disorders

- Blastomeres
Current Benefits and Limitations

- Aneuploidy
- Structural chromosome aberrations
- Single gene disorders
- Mitochondrial disorders
Aneuploidy

- Fluorescence in situ Hybridization (FISH) on interphase nuclei
- Comparative genomic hybridization (CGH) on metaphase chromosomes
FISH Limitations

- Single “spot-check” to test for the presence of a specific chromosome
- Cells are in interphase
- Limited fluorochromes
- Reduced accuracy with additional probes
- Fixation/nuclear spreading
- ~ 10-12 chromosomes tested
Controversies for PGS

• Does aneuploidy screening work?
    • 8 chromosomes assessed
    • Showed no improvement in implantation
  – BUT ……
    • Maternal age (35-41)
      – We wouldn’t offer PGS
    • Many 4-cell embryos biopsied
    • 20% no fluorescence in situ hybridization (FISH) results
    • Failed to test for chromosomes 22 and 15 (~ 25% of aneuploidy)
    • Tested for chromosome 1 ???
    • Biopsied 2 cells sometimes
Controversies for PGS

- PGS doesn’t improve clinical pregnancy rates (gestational sac and fetal heart beat) and delivery rates
  - ASRM Practice Statement, October 2007
  - ESRE Practice Statement – July 2008
  - ACOG – May 2009
  - No prospective, randomized studies to date
CGH on Metaphase Chromosomes

A
1. Isolation of whole-genomic tumor DNA labeled with FITC
2. Isolation of whole-genomic reference DNA labeled with TRITC
3. COT-1 DNA

B
Hybridization 48-72 hours to normal metaphase chromosomes

C
1. Gain of DNA shifts color to green
2. Loss of DNA shifts color to red

Metaphase Chromosome Ratio Profile

density of intensity (green/red) threshold

green regions: amplified in tumor
red regions: deleted in tumor
yellow regions: normal copy-number
CGH Benefits and Limitations

• Tests for all 23-pairs of chromosomes

• Limitations – hybridization takes ~ 3 days and requires an FET
What are the Issues / Risks of PGS?

- Test Limitations
  - FISH - Limited chromosomes
  - CGH – requires an FET

- Embryo mosaicism?

- Damaging the embryo during the biopsy?
  - Reduced implantation?
  - Biochemical pregnancy?
What are the Issues / Risks of PGS?

- Embryo correction of day-3 embryo?
  - CVS – Placental aneuploid colonies
Biopsy a Better Cell Type?

- Trophectoderm
What are the Issues / Risks of Trophectoderm Biopsy and PGS?

- Mosaicism?
  - Inner cell mass
  - Trophectoderm
n = 33 cases
FISH for Chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X and Y

- Patient recruitment – 3+ blasts
- Indication for PGS
- 24 hr turnaround from thaw to transfer
- 114 blasts thawed
- 78% blast survival
- 67% re-expansion
- 83% were biopsied
- 100% PGD results
- PGS (10 chromosomes) - 41% normal, 59% abnormal
- Biopsied cells (mean = 5/patient)
n = 33 cases

3 had no transfer
70% (21/30) + FHT with gestational sac (all > 12 weeks)
3% (1/30) Biochemical
6 normal deliveries
No miscarriages

Blastocyst biopsy and PGS may be a less-invasive and more beneficial option to day-3 blastomere biopsy and PGS
Structural Chromosome Imbalances

Fluorescence *in situ* Hybridization (FISH)

- Reciprocal translocations
  - Telomere clones
  - Genetic balance
    - May have the translocation
  - Breakpoint clones
    - No translocations

- Robertsonian translocations
  - Locus specific clones
  - Genetic balance
    - May have the translocation
Structural Chromosome Disorder

- **Pericentric inversions**
  - Telomere probes
  - Identify duplication/deficient chromosomes in embryos

- **Paracentric inversions**
  - Centromere probes
  - Telomere probes
  - Identify dicentric chromosomes in embryos
Single Gene and Mitochondrial Disorders

- Single gene disorders
  - PCR
  - DNA sequencing
  - Linkage analysis

- Mitochondrial
  - Recessive
Segregation Patterns

**Autosomal Dominant Inheritance**

<table>
<thead>
<tr>
<th>Affected</th>
<th>Not Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dn</td>
<td>nn</td>
</tr>
<tr>
<td>Dn</td>
<td>nn</td>
</tr>
</tbody>
</table>

**KEY**

D = dominant gene  n = normal gene

**Carrier Father**  **Carrier Mother**

<table>
<thead>
<tr>
<th>Gg</th>
<th>Gg</th>
<th>Gg</th>
<th>gg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Carrier</td>
<td>Carrier</td>
<td>Affected</td>
</tr>
</tbody>
</table>

**Carrier Mother**  **Normal Father**

<table>
<thead>
<tr>
<th>xX</th>
<th>xX</th>
<th>xY</th>
<th>xX</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL male</td>
<td>NORMAL female</td>
<td>AFFECTED male</td>
<td>CARRIER female</td>
</tr>
</tbody>
</table>
Single Gene and Mitochondrial Analyses

Limitations

- Single Gene Disorder
  - Mutation must be known
  - Allele dropout – misdiagnosis

- Mitochondrial Disorders
  - Few offered
New Technologies- Microarrays

- Single nucleotide polymorphisms (SNPs)
  - More dense
    - Various density arrays
      - Up to ~ 1,000,000 genomic hits

- CGH
  - Less dense
  - Ratio
  - Oligonucleotides or BACs
    - ~ 42,894 genomic hits
      - ~ Exonic (16404), Intronic (19805), Intergenic (6685)
What are SNPs?

1. Single Nucleotide Polymorphism
2. Normally occurring genetic variant
3. 10,000,000 estimated to be in human genome
4. Stable
5. Used in genetics research to tag genomic segments

5’-ACTGGGAATCCCCGAAGTGTGTGATTACA-3’

DNA segment

5’

DNA variant 1
T

recombination hot spot

5’

DNA variant 2
C

recombination hot spot

3’
CGH

**Conventional CGH**
- Label patient DNA with Cy3
- Label control DNA with Cy5
- Mix
- Cy3/Cy5 ratio >1: Duplication
- Cy3/Cy5 ratio <1: Deletion

**Genome Based Arrays**
- Label patient DNA with Cy3
- Label control DNA with Cy5
- Mix
- Hybridize DNA to genomic clone microarray
- Analyze Cy3/Cy5 fluorescence ratio of patient to control
- Cy3/Cy5 ratio >1: Duplication
- Cy3/Cy5 ratio <1: Deletion
## SNP Arrays vs CGH Arrays

<table>
<thead>
<tr>
<th>Genetic Diagnostics and Screening</th>
<th>SNP</th>
<th>CGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>23/24 chromosome aneuploidy</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Copy number variations (CNVs)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Structural chromosome imbalances</td>
<td>~1.5kb</td>
<td>~1-10mb</td>
</tr>
<tr>
<td>Genome-wide scans</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>What embryo implanted?</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>What partner provided the extra chromosome?</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Single gene disorders</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial mutations</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genetic Diagnostics and Screening</th>
<th>SNP</th>
<th>CGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uni-parental disomy</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Copy neutral event</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
FISH vs Microarrays

FISH: hybridization of a long oligonucleotide to a peri-centromeric or locus specific location
- detection via microscopy
- analysis and interpretation via human

SNP or CGH: DNA markers throughout the chromosome
- CGH is a less dense array
- detection via microarray and scanner
- analysis and interpretation via algorithm
Correlate FISH and Microarray Analysis

- **FISH**
  - Reported world-wide mis-dx is 6-11%
  - Some have suggested that FISH has a ~ 50% mis-dx rate !!!  NO NO NO
    - Based upon rehybridizing completed PGD cases
    - Estimations
    - FISH experience
Materials and Methods

- n = 565 single cells (565 blastomeres (61 day-3 abnormal embryos) and 34 cell lines
- Embryo biopsy of a single cell – laser
  - Blastomere
- Modified whole genome amplification (WGA)
Experimental Problem to Overcome

~ 6 picograms DNA
Need ~ 250,000 pg for microarray analysis

PCR
- Artifacts
- Amplification
- Allele dropout
Modified Whole Genome Amplification (WGA)

- Home Brew
- Parental DNA not required for PGS
Materials and Methods

- Invariant DNA genomic loci to ensure the entire genome was amplified
- TaqMan PCR to ensure heterozygous allele amplification
- Illumina HumanHap370 ~370,000 SNPs
Materials and Methods

- Two-channel intensity values – high-resolution copy-number profile
  - Identify copy number variations (CNVs)

- Genome-wide scans
  - Modified DNA fingerprinting / genotyping
  - Embryo and parental DNA
    - Who provided the extra chromosome?
    - What embryo implanted?

- deCode genetics Disease Minor Professional, Illumina BeadStudio, GenomeStudio and KaryoStudio software
Microarray

Illumina Human HapMap370  ~370,000
Genomic Coverage on CGH Microarray
Results

- Preimplantation blastomeres (n=565 cells from 61 embryos) and cell lines (n=34)
- DNA yields of 700-800 ng / 4-16 hrs reaction
- In many cases, a genomic coverage > 98% (Range 30-98%)
  - Correlates with day-3 embryo quality
- Heterozygous allele detection rate > 90%
| Project       | AMP_Rate | Scanner | Date_Scan | Index | Sample ID   | Call Rate | Gender | p05 Grm | p50 Grm | p95 Grm | p05 Red | p50 Red | p95 Red | p10 GC  | Rep Error Rate | PC Error Rate | PPC Error Rate |
|--------------|----------|---------|-----------|-------|-------------|-----------|--------|---------|---------|---------|---------|---------|---------|---------|--------|----------------|---------------|----------------|
| Training     | W0002392-MSA1 | Control | 1         | 1     | 0895336     | Female    | 197    | 14276  | 29896  | 1803   | 9021   | 25456   | 29362   | 1803   | 9021   | 25456 | 1.7651 | 1.7651 | 1.7651 | 1.7651 |
| Training     | W0002392-MSA1 | Control | 2         | 2     | 08955499    | Female    | 1495   | 18385  | 37541  | 11952  | 11695  | 27934   | 27934   | 11952  | 11695  | 27934 | 1.7651 | 1.7651 | 1.7651 | 1.7651 |
| Training     | W0002392-MSA1 | Control | 3         | 3     | 08950015    | Female    | 917    | 14276  | 29896  | 1803   | 9021   | 25456   | 29362   | 1803   | 9021   | 25456 | 1.7651 | 1.7651 | 1.7651 | 1.7651 |
| Training     | W0002392-MSA1 | Control | 4         | 4     | 08955473    | Female    | 1532   | 15633  | 34628  | 1033   | 10099  | 25657   | 25657   | 1033   | 10099  | 25657 | 1.7651 | 1.7651 | 1.7651 | 1.7651 |
| Training     | W0002392-MSA1 | Control | 5         | 5     | 08959767    | Female    | 944    | 14216  | 34641  | 813    | 9039   | 24729   | 24729   | 813    | 9039   | 24729 | 1.7651 | 1.7651 | 1.7651 | 1.7651 |
| Training     | W0002392-MSA1 | Control | 6         | 6     | 08953339    | Female    | 1008   | 6441   | 73540  | 874    | 741    | 2641    | 2641    | 874    | 741    | 2641 | 1.7651 | 1.7651 | 1.7651 | 1.7651 |
| Training     | W0002392-MSA1 | Control | 7         | 7     | 08959767    | Female    | 122    | 239    | 672    | 74     | 123    | 312     | 312     | 74     | 123    | 312 | 1.7651 | 1.7651 | 1.7651 | 1.7651 |
| Training     | W0002392-MSA1 | Control | 8         | 8     | NA1213      | Female    | 1415   | 18327  | 35619  | 1143   | 11585  | 28377   | 28377   | 1143   | 11585  | 28377 | 1.7651 | 1.7651 | 1.7651 | 1.7651 |
Results

- Microarray detection rate > 90% (some cases > 99%)
  - (Range 30-98%)
    - Correlates with day-3 embryo quality
    - 30% detection rate still permitted aneuploidy detection

- Genotype call rate > 90% (some cases > 99%)
  - (Range 30-98%)
    - Correlates with day-3 embryo quality
    - 30% call rate still permitted aneuploidy detection
Aneuploidy Results

- 23-chromosome molecular karyotype was obtained from all 565 blastomeres and 34 cell lines
  - Blastomeres >25,000 individual chromosomes
  - Cell lines >1564 individual chromosomes
Two Copies of Chromosome 1
Two Copies of Chromosome 18
Three Copies of Chromosome 22
Three Copies of Chromosome 3 (Noise Due to Poor Embryo Quality)
Two Copies of Chromosome 18
Two Copies of Chromosome 21
Three Copies of Chromosome 12
One is Deleted from Band q14?
8p Deletion
23-Chromosome Aneuploidy Results

- 61 embryos (day-3 PGS abnormal for 10-chromosome FISH)
  - 69% (42/61) = mosaic diploid / aneuploid
    - 2-7 Chromosomes
  - 25% (15/61) = mosaic aneuploid
    - 3-9 Chromosomes
  - 7% (4/61) = complex mosaic
    - 3-13 Chromosomes
Results

- Structural chromosome imbalances were identified from all 9 cytogenetically abnormal cell lines
  - del(8q), add(17p), del(17p), add(4q), add(9p), add(14q), dup(18p), dic(5), del(12p) and del(9p)
  - Based upon the density of the SNP microarray
  - CGH array couldn’t identify genetic imbalances
Copy Number Variations

- A high-resolution copy-number map identified CNVs in all 61 embryos and cell lines
  - Inheritable

- Segmental deletions

- Duplications
Copy Number Variations
Genomewide Scans / Molecular Genetic Sequences

- Beckwith-Wiedmann Syndrome
- Some forms of Prader Willi / Angelman Syndrome
- DiGeorge Syndrome
- Some forms of Autism
- Uniparental Disomy
- Single Gene Disorders
- etc
DiGeorge Syndrome
Wilm’s Tumor
Results

- **Modified DNA fingerprinting / genotyping**
  - What embryo implanted?
  - Who provided the extra chromosome?
Conclusions

Chromosomes

- Complete molecular karyotype for all 23/24-pairs of chromosomes
- Genetic imbalances due to reciprocal or Robertsonian translocations, pericentric and / or paracentric inversions
- Duplications (i.e Charcot-Marie-Tooth, type 1A)
- Microdeletion syndromes (i.e. DiGeorge syndrome)
- Using modified microarray and FISH, identify cryptic sub-telomeric rearrangements
Conclusions

• Using Genome-wide scans and modified DNA fingerprinting / genotyping
  • What partner provided the extra chromosome?
  • What embryo implanted?
  • Select best embryo for elective single embryo transfer (eSET)
Conclusions

- Genome wide scans / Molecular Genetic Sequences
  - Complex genetic disorders
  - Single gene disorders
Conclusions – SNP Microarrays

• Analyze polar bodies, blastomeres or trophectoderm cells
Shady Grove Center for Preimplantation Genetics and
Shady Grove Fertility Reproductive Science Center

- Rasmei Pen
- Andy Benner
- Adam Kittai
- Andrew Siegel
- Eric Widra
- Richard Leach
- William G Kearns
Contact Information

• William G. Kearns
  – 301-545-1260
  – william.kearns@integramed.com