Prevention of lysosomal disorders by prenatal genetic diagnosis (PGD) followed by establishing stem cell lines

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EWGGD Paris, June 2012
Disclosure
EWGGD Paris, June 2012

- Nothing to disclose
Prenatal diagnosis for at-risk couples

<table>
<thead>
<tr>
<th>Prenatal Diagnosis</th>
<th>Prevent pregnancy of an affected embryo:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Chorionic villus sampling (CVS) at 11-13wks</td>
<td>• Decision not to have children</td>
</tr>
<tr>
<td>• Amniocentesis at 16-20wks</td>
<td></td>
</tr>
<tr>
<td>• Fetal blood sampling (FBS) &gt;22 weeks</td>
<td>• Preimplantation genetic diagnosis (PGD)</td>
</tr>
</tbody>
</table>
PGD background

- First PGD baby born in 1989

- PGD for single gene disorders have been performed for hundreds of different single gene diseases.

- PGD can be performed for any genetic condition for which a known genetic basis.
Why PGD?

- Desire to avoid pregnancy termination of affected embryos
- Moral / religious objections to abortion
- Repeated miscarriage due to chromosomal abnormalities
- HLA matching
- Prevention of late onset and non-severe diseases
Limitations of PGD

- Requires *In Vitro Fertilization* (IVF) with intracytoplasmic sperm injection (ICSI)

- Increased risk (from IVF and ICSI)
  - birth defects (3% → 4.5%) (NEJM, May 2012)
  - low birth weight
  - imprinting defects (Beckwith Wiedemann and Angelman syndrome)
Stages of PGD

1. Egg donor is given fertility drugs
2. Multiple eggs are produced
3. Embryos are analyzed for genetic defects
4. Eggs are fertilized to produce embryos
5. Only healthy embryos are injected into uterus
6. Mother gives birth to genetically healthy baby
Biopsy options

Polar Bodies 1 and 2
Day 0 and 1

Blastomere
Day 3

Trophectoderm (blastocyst biopsy)
Day 5
Polar bodies extruded by oocyte during meiosis I and II

For maternal autosomal dominant or X-linked diseases
Intact blastomere with a well-defined single nucleus
Allele drop out (ADO)-preferential amplification in single cells can lead to misdiagnosis.
PGD accuracy depends on analysis of polymorphic genetic markers
Gaucher haplotype

CA 230 228
GT 237 240
AAAG 195 194

Mutation IVS2+1 WT
D1S2140 250 250
D1S2721 243 241

N370S WT
D1S2721 243 241

WT-wild type
Lysosomal disorders for which we performed PGD

- Gaucher disease type 1 (phenotype was predicted to be severe based on the mutations of the parents) (Autosomal recessive)

- Mucopolysaccharidosis Type II (Hunter syndrome) (X linked)

- Fabry disease (X linked)

- Tay Sachs disease (Autosomal recessive)
## PGD for Gaucher type 1 disease

<table>
<thead>
<tr>
<th>Family</th>
<th>Mutation</th>
<th>PGD no.cycles</th>
<th>Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IVS2+1/N370S*</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>84GG/ homozygote N370S</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>84GG/1604G&gt;A</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>84GG/R535Q</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

* also compound heterozygote for Tay Sachs HEXA gene mutation
Family 1.
Tay Sachs and Gaucher

- Tay Sachs carrier
- Gaucher carrier

25% Tay Sachs + 25% Gaucher

9/16 embryos for transfer
Family 1

- Carriers for both Gaucher and Tay Sachs diseases
- Simultaneous PGD for two monogenic disorders
- Simultaneously analyzed 14 markers on a single cell from each embryo
- Three separate PGD cycles resulted in three healthy children
Family 2

- Female homozygous N370S
- Husband carrier of 84GG

- No embryo will be wild type
- All embryos are obligate maternal carriers
Family 2 – One PGD cycle

<table>
<thead>
<tr>
<th>PGD cycles</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>oocytes retrieved</td>
<td>10</td>
</tr>
<tr>
<td>oocyte fertilized</td>
<td>7</td>
</tr>
<tr>
<td>embryos analyzed</td>
<td>7</td>
</tr>
<tr>
<td>embryos diagnosed</td>
<td>7</td>
</tr>
<tr>
<td>embryos carriers</td>
<td>4</td>
</tr>
<tr>
<td>embryos mutant</td>
<td>3</td>
</tr>
<tr>
<td>embryos transferred</td>
<td>2</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Birth of a healthy boy</td>
</tr>
</tbody>
</table>
Family 3 – 84GG/1604G>A
One PGD cycle

<table>
<thead>
<tr>
<th>PGD cycles</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>oocytes retrieved</td>
<td>7</td>
</tr>
<tr>
<td>oocyte fertilized</td>
<td>6</td>
</tr>
<tr>
<td>embryos analyzed</td>
<td>6</td>
</tr>
<tr>
<td>embryos diagnosed</td>
<td>6</td>
</tr>
<tr>
<td>embryos wild type</td>
<td>2</td>
</tr>
<tr>
<td>embryos mutant</td>
<td>4</td>
</tr>
<tr>
<td>embryos transferred</td>
<td>2</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Birth of a healthy boy</td>
</tr>
</tbody>
</table>
Family 4

- Female Ashkenazi Jewish origin carrier of N370S
- Husband –Caucasian male carrier of a private mutation R535Q
- One daughter affected that died at age 5 after severe pulmonary involvement and lung transplant
- Spontaneous pregnancy-CVS
  - Affected embryo, termination of pregnancy
Family 4-cont

4 PGD cycles

Pregnancy achieved

Spontaneous abortion week 10

4 PGD cycles

Healthy boy born

2 PGD cycles

Twin pregnancy ongoing week 25
Overall Take home baby rate / family: 85%

<table>
<thead>
<tr>
<th>Disease</th>
<th>No Families</th>
<th>Mean No. PGD cycles to Pregnancy</th>
<th>No PGD cycles of Polar body only</th>
<th>Children born</th>
<th>human embryonic stem cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunter*</td>
<td>4</td>
<td>2.5</td>
<td>4</td>
<td>4 + 1 preg</td>
<td>1</td>
</tr>
<tr>
<td>Gaucher**</td>
<td>4</td>
<td>1.5</td>
<td>0</td>
<td>6 + twin preg</td>
<td>1</td>
</tr>
<tr>
<td>Fabry</td>
<td>2</td>
<td>1.2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Tay Sachs**</td>
<td>11</td>
<td>3.2</td>
<td>1</td>
<td>8 + preg</td>
<td></td>
</tr>
</tbody>
</table>

* 1 couple combined for Hunter and Albinism
** 1 couple combined for Tay Sachs and Gaucher
Direct derivation of HESCs from LSD (Hunter and Gaucher) embryos

- Allows establishing pluripotent stem cell lines with naturally occurring mutations
- Depends on availability of diseased embryos

- Hunter: XX del exon 4-8
- Gaucher: XY 84GG/1604G>A
Direct derivation of HESCs from Hunter and Gaucher diseased embryos

- Gaucher XY 84GG/1604G>A

Future plans: differentiation of the Gaucher stem cell line into neuronal cells to study the effect of the 84GG mutation on neurodegeneration.
RT-PCR for pluripotent associated markers

<table>
<thead>
<tr>
<th>OCT4</th>
<th>SOX2</th>
<th>Nanog</th>
<th>REX1</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><img src="image1.png" alt="Image of RT-PCR results" /></td>
<td></td>
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</tr>
</tbody>
</table>

Tra-1-60 and SSEA3

Karyotype of the Hunter line (passage 8)

<table>
<thead>
<tr>
<th>File Name</th>
<th>Label</th>
<th>Events</th>
<th>% Gated</th>
<th>% Total</th>
<th>X Mean</th>
<th>Y Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunter PE+FITC.004</td>
<td>UL</td>
<td>2276</td>
<td>8.14</td>
<td>4.55</td>
<td>15.73</td>
<td>206.44</td>
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<tr>
<td>Hunter PE+FITC.004</td>
<td>UR</td>
<td>24223</td>
<td>86.59</td>
<td>48.45</td>
<td>145.94</td>
<td>261.06</td>
</tr>
<tr>
<td>Hunter PE+FITC.004</td>
<td>LL</td>
<td>387</td>
<td>1.38</td>
<td>0.77</td>
<td>12.75</td>
<td>19.99</td>
</tr>
<tr>
<td>Hunter PE+FITC.004</td>
<td>LR</td>
<td>1087</td>
<td>3.89</td>
<td>2.17</td>
<td>112.72</td>
<td>23.2</td>
</tr>
</tbody>
</table>
Conclusions

- PGD is an accurate and reliable alternative to pregnancy terminations for couples who are carriers or affected with a genetic disorder.

- Of ~200 born PGD children in our unit, 10% were born to carriers of lysosomal disorders.

- PGD allows derivation of human embryonic stem cells from diseased embryos which can be used as a valuable source for research of pathogenesis and drug testing.
• Prof. Talia Eldar Geva
• Prof. Ehud Margalioth
• Dr. Baruch Brooks
• Irit Varshaver
• Aron Perez

• Prof. Ephrat Levy-Lahad
• Dr. Pinhas Renbaum
• Dr. Rachel Beeri
• Dr. David Zeevi
• Sharon Zeligson
• Shira Perlberg
• Dr. Yaffa Nevo
• Elina Farhi
• Merav Ben-Shlomo
• Shira Shaviv
• Miriam Pat
• Hagit Elharar
• Nava Biton
• Orit Lobel

Gaucher unit

Prof. A Zimran
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