The Transcriptional Repressor BCL6 is a Diagnostic Biomarker for Endometriosis and Related Infertility

Bruce A. Lessey, Emily Evans-Hoeker, Ling-Wen Yuan, Jae-Wook Jeong, David P Schammel, Steven L Young

Greenville Health System, Greenville SC, Michigan State University, Grand Rapids, MI, University of North Carolina at Chapel Hill, Chapel Hill, NC

SEUD Congress, Saturday May 8th, 2015
Disclosures

• Abbvie
• Pfizer
• Sepal, Inc.
Endometrium

- Cycles throughout the reproductive lifetime
- Regulated by estrogen and progesterone
- Reflects the immunologic milieu of the host
- Is disrupted in women with endometriosis
Implantation

• Requires a receptive endometrium
• Progesterone action is essential
• Timing is important for success (synchrony)
Background on Pregnancy

Adapted from Macklon et al, Human Reproduction, 2002
Endometriosis

- Affects 176 million women
- Costs $22 billion per year in US alone
- Delays in diagnosis are common
- Associated with infertility
- Laparoscopy as the gold standard, has an unacceptable specificity
Kim et al, HR 2015
BCL6

- B-cell lymphoma protein-6 (BCL6) was identified as upregulated in normal mid-secretory endometrium (Talbi et al., 2006)
- It is a gene repressor involved in inactivation of p53, FOX01, IHH pathway (GLI1)
- Inhibited by STAT5 but promoted by STAT3
- Markedly up-regulated in the endometrium of women with endometriosis
Methods

- BCL6 was measured by IHC and western blot in normal and abnormal endometrium
- Test set of women undergoing laparoscopy was studied
- Prospective assessment of women with UI was undertaken comparing these findings to a prospectively obtained fertile control group
Western Blot

BCL-6 level in Human Endometriosis samples

<table>
<thead>
<tr>
<th></th>
<th>Proliferative</th>
<th>Secretory</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Endometriosis</td>
</tr>
<tr>
<td>BCL6</td>
<td></td>
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<tr>
<td>Actin</td>
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</table>
Immunohistochemistry
Initial Test Set

- Sensitivity = 96%
- Specificity = 93%
- PPV = 96%
- NPV = 93%

p < 0.0001
### Demographics

<table>
<thead>
<tr>
<th>Characteristic (mean ± SD)</th>
<th>Fertile Control Group (n=28)</th>
<th>Unexplained Infertility (n = 121)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>32.77 ± 2.6</td>
<td>32.5 ± 4.1</td>
</tr>
<tr>
<td>BMI</td>
<td>25.2 ± 4.5</td>
<td>24.93 ± 4.9</td>
</tr>
<tr>
<td>Parity</td>
<td>1.65 ± 0.5</td>
<td>0.2 ± 0.56 (p &lt; 0.001)</td>
</tr>
<tr>
<td>HSCORE</td>
<td>0.4 ± 0.68</td>
<td>3.1 ± 0.96 (p &lt; 0.001)</td>
</tr>
</tbody>
</table>

Sixty-seven of the UI eventually had laparoscopy after the biopsy
Unexplained Infertility Group

67 had L/S
87% Tested Pos
121 UI

93.4% Endometriosis
4.6% Hydrosalpinges
3 False neg

98% of BCL6 positive subjects had findings. There were 3 false negative tests
Clinical Data

- **BCL6 neg**
  - 4 Pregnant
  - 5 Not Pregnant

- **BCL6 Pos**
  - 6 Pregnant
  - 44 Not Pregnant

**IVF and FET Cycles**
RT-PCR in Ishikawa Cells
Baboon Endometriosis Model
Conclusions

• A single diagnostic biomarker for endometriosis exists
• A majority (>80%) of unexplained infertility patients have endometrial receptivity defects
• BCL6 is a likely candidate to be a root cause of progesterone resistance
• Progesterone resistance associated with endometriosis is mediated by inflammatory cytokines and is reversible
• Endometriosis is likely a primary and important occult cause of IVF failure
Acknowledgement

• Asgerally T. Fazleabas PhD and Jae Wook Jeong PhD – MSU
• Steve Young MD, PhD, UNC
• John Langenheim PhD
• NIH-R01 support

• www.theE2test.com
<table>
<thead>
<tr>
<th>Study</th>
<th>Odds ratio (95% CI)</th>
<th>Percent weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mahadevan et al, '83</td>
<td>1.51 (0.32,7.10)</td>
<td>0.6</td>
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<tr>
<td>Wardle et al, '85</td>
<td>0.52 (0.06,4.85)</td>
<td>0.7</td>
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<tr>
<td>Matson et al, '86</td>
<td>0.36 (0.13,1.01)</td>
<td>2.8</td>
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<tr>
<td>Frydman et al, 87</td>
<td>0.83 (0.39,1.80)</td>
<td>4.0</td>
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<tr>
<td>Inoue et al, '92</td>
<td>1.21 (0.87,1.69)</td>
<td>16.9</td>
</tr>
<tr>
<td>Mills et al, '92</td>
<td>0.94 (0.47,1.86)</td>
<td>4.6</td>
</tr>
<tr>
<td>Simon et al, '94</td>
<td>0.26 (0.12,0.54)</td>
<td>8.0</td>
</tr>
<tr>
<td>Dmowski et al, '95</td>
<td>1.31 (0.72,2.39)</td>
<td>5.0</td>
</tr>
<tr>
<td>Gerber et al, 95</td>
<td>0.84 (0.58,1.20)</td>
<td>18.1</td>
</tr>
<tr>
<td>Olivennes et al, '95</td>
<td>0.93 (0.61,1.41)</td>
<td>12.0</td>
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<tr>
<td>Tanbo et al, '95</td>
<td>0.89 (0.60,1.32)</td>
<td>14.0</td>
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<tr>
<td>Arici et al, '96</td>
<td>0.49 (0.24,1.02)</td>
<td>6.0</td>
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<tr>
<td>Padigas et al, '96</td>
<td>1.64 (0.82,3.30)</td>
<td>3.1</td>
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<tr>
<td>Huang et al, '97</td>
<td>0.68 (0.32,1.46)</td>
<td>4.3</td>
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<tr>
<td>Overall (95% CI)</td>
<td>0.81 (0.72,0.91)</td>
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