MiRacles for babies with abnormal lung development and Congenital Diaphragmatic Hernia

Richard Keijzer, MD, MSc, PhD, FACS
Thorlakson Chair in Surgical Research
Conflict of Interest Disclosure

I hold a patent application (PCT/CA2015/051028) containing technology described in the presentation.

I have filed for a patent to use circular RNAs as biomarkers for abnormal lung development and CDH.
How it began
We don’t do chart reviews here, why don’t you go in the lab for the next few months.
The 'golden circle' from Simon Sinek
Mortality: >400,000 since 2000

Image: Shutterstock
Main problem: Abnormal lungs
What is wrong with CDH lungs?
Can we fix the lungs before birth?
Meta-analysis FETO improves survival in isolated CDH

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>FETO Events</th>
<th>Total</th>
<th>Control Events</th>
<th>Total</th>
<th>Weight</th>
<th>Odds Ratio M–H, Fixed, 95% CI</th>
<th>Odds Ratio M–H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deprest 2004</td>
<td>10</td>
<td>21</td>
<td>1</td>
<td>12</td>
<td>19.5%</td>
<td>10.00 [1.09, 91.98]</td>
<td></td>
</tr>
<tr>
<td>Deprest 2006</td>
<td>12</td>
<td>24</td>
<td>3</td>
<td>37</td>
<td>34.5%</td>
<td>11.33 [2.72, 47.17]</td>
<td></td>
</tr>
<tr>
<td>Peralta 2011</td>
<td>9</td>
<td>28</td>
<td>1</td>
<td>13</td>
<td>27.1%</td>
<td>5.68 [0.64, 50.73]</td>
<td></td>
</tr>
<tr>
<td>Ruano 2011</td>
<td>10</td>
<td>17</td>
<td>1</td>
<td>18</td>
<td>11.7%</td>
<td>24.29 [2.60, 227.25]</td>
<td></td>
</tr>
<tr>
<td>Ruano 2012</td>
<td>10</td>
<td>20</td>
<td>0</td>
<td>21</td>
<td>7.1%</td>
<td>43.00 [2.29, 806.44]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>110</td>
<td></td>
<td>101</td>
<td></td>
<td>100.0%</td>
<td>13.32 [5.40, 32.87]</td>
<td></td>
</tr>
</tbody>
</table>

Total events: 51, 6
Heterogeneity: Chi² = 1.59, df = 4 (P = 0.81); I² = 0%
Test for overall effect: Z = 5.62 (P < 0.00001)

**Meta-Analysis**

Fetal Tracheal Occlusion for Severe Pulmonary Hypoplasia in Isolated Congenital Diaphragmatic Hernia

* A Systematic Review and Meta-analysis of Survival*

*Jamila Al-Maary, MD,* Mary P. Eastwood, MBChB,* Francesca Maria Russo, MD,* Jan A. Deprest, PhD,*§ and Richard Keijzer, PhD*†

*Annals of Surgery* • Volume 264, Number 6, December 2016
But, negative side effects!

https://www.tommys.org/pregnancy-information/pregnancy-complications/waters-breaking-early-pprom
MicroRNAs and Congenital Diaphragmatic Hernia

Unique Tracheal Fluid MicroRNA Signature Predicts Response to FETO in Patients With Congenital Diaphragmatic Hernia

Patricia Pereira-Terra, MSc,† Jan A. Deprest, MD, PhD,‡ Ramin Kholdebarin, MD, MSc,* Naghmeh Khoshgooy, MS,* Philip DeKoninck, MD, PhD,‡ Anne A. Boerema-De Munck,§ Jinxia Wang,¶ Fuqin Zhu,* Robbert J. Rottier, PhD,§ Barbara M. Iwasiow, MSc,* Jorge Correia-Pinto, MD, PhD,† Dick Tibboel, MD, PhD,§ Martin Post, DVM, PhD,* and Richard Keijzer, PhD*

Annals of Surgery • Volume 262, Number 6, December 2015
Higher miR-200b has better outcomes
Epithelial-to-Mesenchymal Transition (EMT)

Epithelial cells

Mesenchymal cells
MicroRNA-200b regulates distal airway development by maintaining epithelial integrity

Naghmeh Khoshgoo1,2,3, Robin Visser1,2, Landon Falk1,2, Chelsea A. Day1,2, Dustin Ameis1,2, Barbara M. Iwasiow1,2, Fuqin Zhu1,2, Arzu Öztürk4,5, Sujata Basu1,3, Molly Pind4,5, Agnes Fresnosa6,7, Mike Jackson6, Vinaya Kumar Siragam1,2, Gerald Stelmac1,3, Geoffrey G. Hicks4,5, Andrew J. Halayko1,3 & Richard Keijzer1,2,3
miR-200b +/- mice have higher lung tissue damping and elasticity

* P< 0.05, ** P< 0.01, *** P< 0.001
miR-200b -/- lungs are hypoplastic
miR-200b -/- lungs have more vimentin

wt

miR-200b -/-
Wildtype

miR-200b −−

Unpublished results
miR-200b knockout mice have pulmonary hypertension on cardiac echography

**Unpublished results**
miR-200b-/- lungs have thicker vessel walls

Unpublished results
miR-200b-/- lungs have thicker vessel walls

Unpublished results
miR-200b-/- lungs have thicker vessel walls

Unpublished results
miR-200b-/- lungs have thicker vessel walls

Unpublished results
NITROFEN MODEL OF CDH

Nitrofen

E0 E9 E11 E14 E17 E22

Lung Diaphragm Fetal breathing Birth

80% CDH 100% PH
Prenatal microRNA miR-200b Therapy Improves Nitrofen-induced Pulmonary Hypoplasia Associated With Congenital Diaphragmatic Hernia

Naghmeh Khoshgoo, MSc,* Ramin Kholdebarin, MD, MSc,* Patricia Pereira-Terra, PhD,*†
Thomas H. Mahood, MSc,* Landon Falk, BSc,* Chelsea A. Day, BSc,* Barbara M. Iwasio, MSc,*
Fuqin Zhu, BSc,* Drew Mulhall, BSc,* Carly Fraser, BSc,* Jorge Correa-Pinto, MD, PhD,††
and Richard Keijzer, MD, PhD, MSc, FACS*

In Situ Hybridization
Control Hypoplastic+ CDH

PCR (E21)

Blue staining: miR-200b
MiR-200b & Human Bronchial Epithelial Cells

* P< 0.05

* * P< 0.05

* * P< 0.05
miR-200b maintained epithelial cell phenotype in bronchial epithelial cells

Control

MiR-200b inhibitor

Green: Epithelial Marker (cytokeratin)
Red: Mesenchymal Marker (Vimentin)
miR-200b expression is high at branching lung tips
miR-200b improves branching hypoplastic lungs

Normal lungs

Hypoplastic lungs

Control

200b inhibitor

Hypoplastic

Hypoplastic + miR-200b

* P< 0.05, ** P< 0.01

miR-200b improves branching hypoplastic lungs

Number of peripheral airway buds (D4/D0)

Normal lungs

Hypoplastic lungs

* P< 0.05, ** P< 0.01

Annals of Surgery • Volume 269, Number 5, May 2019
Prenatal miR-200b improves lung hypoplasia

- hypoplastic
  - (80% CDH)
- hypoplastic +
  - miR-200b
  - (15% CDH)
Prenatal miR-200b reduces CDH incidence
Prenatal miR-200b improves lung hypoplasia
Prenatal miR-200b improves lung hypoplasia
Prenatal miR-200b improves lung hypoplasia

Surface Density

Annals of Surgery • Volume 269, Number 5, May 2019
Proteome suggests that nitrofen-induced abnormal lung development is an immune disease?

*Unpublished results*
Proteome suggests that nitrofen-induced abnormal lung development is an immune disease?

Unpublished results
## Upregulated nodes KEGG

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Total</th>
<th>Expected</th>
<th>Hits</th>
<th>P.Value</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epstein-Barr virus infection</td>
<td>114</td>
<td>0.458</td>
<td>4</td>
<td>0.000973</td>
<td>0.207</td>
</tr>
<tr>
<td>Cell cycle</td>
<td>127</td>
<td>0.51</td>
<td>3</td>
<td>0.0136</td>
<td>0.75</td>
</tr>
<tr>
<td>Phagosome</td>
<td>49</td>
<td>0.197</td>
<td>2</td>
<td>0.0161</td>
<td>0.75</td>
</tr>
<tr>
<td>Notch signaling pathway</td>
<td>49</td>
<td>0.197</td>
<td>2</td>
<td>0.0161</td>
<td>0.75</td>
</tr>
<tr>
<td>Wnt signaling pathway</td>
<td>147</td>
<td>0.59</td>
<td>3</td>
<td>0.02</td>
<td>0.75</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>170</td>
<td>0.682</td>
<td>3</td>
<td>0.0293</td>
<td>0.75</td>
</tr>
<tr>
<td>Salmonella infection</td>
<td>68</td>
<td>0.273</td>
<td>2</td>
<td>0.0299</td>
<td>0.75</td>
</tr>
<tr>
<td>Long-term potentiation</td>
<td>69</td>
<td>0.277</td>
<td>2</td>
<td>0.0307</td>
<td>0.75</td>
</tr>
<tr>
<td>Phenylalanine, tyrosine and tryptophan biosynthesis</td>
<td>8</td>
<td>0.0321</td>
<td>1</td>
<td>0.0317</td>
<td>0.75</td>
</tr>
<tr>
<td>Fc epsilon RI signaling pathway</td>
<td>75</td>
<td>0.301</td>
<td>2</td>
<td>0.0358</td>
<td>0.762</td>
</tr>
<tr>
<td>ErbB signaling pathway</td>
<td>87</td>
<td>0.349</td>
<td>2</td>
<td>0.0469</td>
<td>0.829</td>
</tr>
<tr>
<td>Malaria</td>
<td>12</td>
<td>0.0482</td>
<td>1</td>
<td>0.0472</td>
<td>0.829</td>
</tr>
</tbody>
</table>
Does EBV cause CDH??????
Does EBV cause CDH??????
ABOUT WiSDOM.

WiSDOM is all about children with congenital anomalies. A congenital anomaly is a birth defect. Some babies are born with a congenital anomaly that requires surgery immediately after birth. Until a few decades ago, most of these babies died. Recent improved surgical and intensive care techniques have resulted in better survival. Currently, we do not know how these babies do in life when they grow up. To find out, we created a database of almost 800 surgical congenital anomaly patients, recording birth and surgery details. We plan to link our surgical database to population data managed by the Manitoba Centre for Health Policy (MCHP). MCHP databases contain unique information about healthcare, education, and social service utilization for all Manitobans. This linkage will allow us to answer the following questions:

1. How many children have congenital anomalies requiring surgery?
2. How do children with congenital anomalies do at school and in life compared to children without a defect?
3. Why do some children with surgical congenital anomalies do better than others with congenital anomalies?

The answers to these questions will help us understand the causes and long-term outcomes of surgical birth defects and improve the care of babies born with surgical congenital anomalies.

OBJECTIVES:

1. WHO is at risk: Identify maternal factors associated with surgical congenital anomalies: Maternal demographic information will be identified using MCHP databases to determine the populations with highest risk of having a baby with congenital anomalies.

2. HOW are children doing: Compare the long-term outcomes of patients with surgical congenital anomalies to those of age-matched controls: Virtual long-term follow-up of each patient in WiSDOM using MCHP linkages to determine their medical, educational, psychosocial, socioeconomic outcomes and comparing them with the age-matched control from MCHP.

3. WHY do some children do better: Determine patient and maternal factors that affect long-term patient outcomes: After defining the maternal risk factors and long-term outcomes for the WiSDOM patient cohort, subgroups who have the highest risk of poor outcomes will be identified, as well as the demographic, medical, educational, psychosocial and socioeconomic determinants of favourable long-term outcomes.

HYPOTHESIS:

WiSDOM has established a multidisciplinary long-term follow-up clinic at the Children’s Hospital for patients with surgical congenital anomalies. Children attending the clinic receive evidence-based follow-up for early identification and intervention for patients and families. For many, early intervention will prevent complications and improve outcomes. The WiSDOM clinic includes improved and coordinated care, ongoing education, increased quality of life, and ease of access to health care professionals.

TEAM:

The WiSDOM team includes expertise in:
- Pediatric surgery
- Neonatology
- Pediatrics
- Allied health care
- Pediatric medicine
- Child health outcomes
- Biostatistics
- Data analysis

WiSDOM CLINIC:

WiSDOM has established a multidisciplinary long-term follow-up clinic at the Children’s Hospital for patients with surgical congenital anomalies. Children attending the clinic receive evidence-based follow-up for early identification and intervention for patients and families. For many, early intervention will prevent complications and improve outcomes. The WiSDOM clinic includes improved and coordinated care, ongoing education, increased quality of life, and ease of access to health care professionals.

SIGNIFICANCE:

Surviving congenital abnormalities has drastically improved, therefore, we need to refocus our attention on optimizing long-term medical, educational, psychosocial and socioeconomic outcomes. By linking our surgical congenital anomalies patient cohort with MCHP databases, we will be able to begin this optimization. Linking databases will provide the opportunity for virtual long-term follow-up for the first time in this patient population. This virtual follow-up will identify the risk factors and outcomes of surgical congenital anomalies. This information will guide the development of preventative strategies to provide better care to babies born with surgical congenital anomalies.

DEVOTION:

1. Provides support for prospective data collection and entry for babies born with surgical congenital anomalies after 2016
2. Facilitates consent and enrolment of participants into the WiSDOM study and collection of research data in the WiSDOM Clinic
3. Helps in developing policies to direct risk mitigation strategies
4. Assists in developing a plan to establish a multidisciplinary long-term follow-up clinic for WiSDOM children born in Manitoba within the next 5 years
5. DEVOTION provided initial funding for the retrospective data collection for all babies born in Manitoba from 1991 - 2016 in addition to the funding for prospective data collection and linkages to MCHP.
Does EBV cause CDH?

Infectious Mononucleosis (ICD-9:075)

Odds ratio = 0.49 for mothers with CDH baby compared to controls from the general population

95% CI = 0.12-1.36
P-value = 0.2371
Epithelial-to-Mesenchymal Transition, CDH and EBV

Epithelial cells

Mesenchymal cells

?
CircularRNA

- More stable, more abundant and specific than linear RNAs.
- Regulate gene expression at transcriptional and post-transcriptional level by serving as microRNA sponges and interacting with mRNA and proteins.

http://www.med.upenn.edu/wiluszlab/research.html

Circular RNAs

- Upstream regulators of miRNAs (epigenetic regulation)
- “Head-to-tail” splicing

Ideal Biomarkers:

- Stable
- Conserved
- Tissue- and development stage-specific expression
- High abundance in extracellular compartments
Circular RNA profile is dysregulated in E21 nitrofen-induced hypoplastic lungs
BaseScope™ ISH

i. Backsplice-specific probe

ii. Adjacent hybridization of probes to circular RNA

iii. Preamplifier binding

iv. Amplification cascade

v. Label probe binding for fluorescent / chromogenic readout
BaseScope™ E21 Rat Lung

(rno_circRNA_007475)
Control Adult Rat Serum: rno_circRNA_007475
CircRNA profile distinguishes CDH lung from control

A

Mid Pregnancy

End Pregnancy

B

Mid Pregnancy PLS-DA

End Pregnancy PLS-DA

Legend

- CDH
- Control

CircRNA profile distinguishes CDH lung from control
CircRNA profile can distinguish FETO survivors from non-survivors

<table>
<thead>
<tr>
<th></th>
<th>Survivors</th>
<th>Non-Survivors</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational Age at Plug (weeks)</td>
<td>28.1 (28.7 - 27.1)</td>
<td>27.9 (29.1 - 27.0)</td>
<td>0.84</td>
</tr>
<tr>
<td>Observed/ Expected Lung to Head Ratio (%)</td>
<td>22.5 (23.6 - 17.5)</td>
<td>21.6 (24.0 - 15.9)</td>
<td>0.87</td>
</tr>
<tr>
<td>Liver herniated</td>
<td>10 (91%)</td>
<td>9 (100%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Fetal gender</td>
<td>7 male/ 4 female</td>
<td>4 male/ 5 female</td>
<td>0.65</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2780 (3180 - 2160)</td>
<td>3195 (3278 - 2650)</td>
<td>0.3</td>
</tr>
</tbody>
</table>
CircRNA profile can distinguish FETO survivors from non-survivors

Unpublished results
Prenatal treatment

FETO
Drugs
Stem cells
Lung abnormalities
Diaphragmatic hernia

Pathogenesis

Prenatal diagnosis

Biomarkers
Imaging

Figure courtesy: Dustin Ameis
Thank you MIRACLA Lab
Acknowledgements

Laboratory members
Nolan Deleon
Chelsea Day
Landon Falk
Andrew Tse
Daywin Patel

Pediatric Surgery
Melanie Morris
Anna Shawyer
Giuseppe Retrosi
Suyin Lum Min
BJ Hancock
Nathan Wiseman
Cindy Holland

Previous Laboratory members
Dustin Ameis
Lojine Ayoub
Carly Fraser
Barbara Iwasiw
Shana Kahnamoui Zadeh
Ramin Kholdebarin
Naghmeh Khoshgoo
Eimear Kirby
Thomas Mahood
Samira Seif
Phillip Snarr
Robin Visser
Fuqin Zhu

UofM collaborators
Geoff Hicks’s group
Andrew Halayko’s group
Malcolm Xing’s group
Neelofeer Mookherjee’s group

Other Collaborators
Robbert Rottier
Dick Tibboel
Martin Post
Jan Deprest
Patrice Eastwood
Francesca Russo
Martin Lacher
Richard Wagner
Jorge Correia-Pinto
Patricia Pereira-Terra

Patients and families
thank you