The use of proteomic for the prediction of adverse pregnancy outcomes

Dr Jenny Myers PhD MRCOG
Clinician Scientist
University of Manchester, UK
Disclosure of interests

- Grant funding Wellcome Trust/Academy Medical Sciences/BBSRC
- Research agreement with Pronota
- Clinical Lead SCOPE study Manchester
- Industrial sponsorship Alere
- Member Maternal Medicine Clinical Studies Group, RCOG
The need for prediction

- Clinical risk factors are not good enough predictors of pregnancy complications
- Selecting patients for preventative treatments is not possible without effective prediction
- Complex diseases → Several biomarkers are necessary

SCOPE Study
Screening for Pregnancy Endpoints on behalf of the SCOPE Consortium
Annual Direct Healthcare Costs To Provide Antenatal Care & Treatment

SCOPE Study
Screening for Pregnancy Endpoints on behalf of the SCOPE Consortium

$41.3 Billion
Total Annual Cost For Diseases of Late Pregnancy in Developed Countries

Neonatal Intensive Care
$25.9 Billion

Maternal Hospitalization
$2 Billion

Caesarean Section
$3.4 Billion

Prenatal Care Visits/Scans
$10 Billion
SCOPE consortium

Develop a unique international pregnancy biobank with

- detailed clinical phenotypes
  - all known and novel clinical risk factors
  - well characterized disease phenotypes
  - comprehensive bank of quality specimens

Use the biobank to develop screening tests for late pregnancy complications based on

- Clinical risk factors with algorithms
- Blood biomarkers with algorithms

SCOPE Study
Screening for Pregnancy Endpoints
on behalf of the SCOPE Consortium
Scope Study

SCOPE Study
Screening for Pregnancy Endpoints on behalf of the SCOPE Consortium

15 ± 1 weeks

20 ± 1 weeks

Pregnancy Outcome

Oct 2010
5400 Women
850,000 Specimens

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SCOPE Clinical Study

**Recruit: 15 weeks**
- Consent, comprehensive clinical data
- Blood and urine specimens
- Specimen processing within 2-3h, multi- aliquot samples (n=70), store -80°C

**2nd Visit: 20 weeks**
- Clinical data, blood, urine and cervical specimens
- Partner’s data and blood specimen

**Ultrasound Scan: 20 and 24 weeks**
- Cervical scan (20w only), uterine and umbilical artery Doppler, fetal growth scan

**End of Pregnancy**
- Tracking pregnancy outcomes
- Late pregnancy biobank- time of disease and healthy pregnancy
- See mothers and babies <48h delivery
- Cord blood or baby buccal smear
**5. Demography**

- **Marital status:** 2-Married
- **Country of Birth:** New Zealand
- **Main Ethnic Origin:** New Zealand-caucasian
- **Other Ethnic Origin:**
- **Ethnicity for customized centiles:** 1-European
- **Imigration History:**
- **Participant Migration:** (number of years ago imigrated)
- **Partner’s Migration:** 1-Not an immigrant
- **Total years of schooling:** 13
- **Tertiary Education:** 2-Graduated (University)
- **Tertiary Education:** 2-Graduated (other)
- **Current job situation:** 1-Full time work
- **Maternal Occupation:** business analyst
- **Major group:** 2-Professionals
- **Socioeconomic Index:** 61
- **Current living situation:** 1-Partner (lives with)
- **Children in household:** YES NO
26. Newborn Data

FOR ALL BABIES (including Intrauterine Deaths)

* Liveborn: YES NO

* Date of Birth: 12/09/2005 (dd/mm/yyyy)

* G.A. at delivery: 36 + 6 (w+d)

* Sex: 2-Female

Apgar Score: 8 9 (1 min, 5 min)

Intubation at birth: 1-No

Cord arterial pH: Base deficit (-): Base excess (+):

Birthweight: 2340 (g) Unknown birthweight

Customised Birthweight Centile: 6

Head circumference: 31.4 (cm)

Length: 49.0 (cm) Measured in noonatometer

Midarm circumference: 9.1 (cm)

Measurements performed by: 1-Research midwife

* Admitted to Neonatal unit: YES NO

* EDTA plasma YES NO 06/09/2006 11:00

* Heparin plasma YES NO 06/09/2006 11:00

* Citrate plasma YES NO 06/09/2006 11:00
### SCOPE biobank

**Patient overview**

- **PetID:** 2070 | **Initials:** TC | **EDD:** 31/03/2007 | **GA at delivery:** 27+3 | **Delivery:** 03/01/2007 | **Control:** NO

### Biobank Management - 15 w Specimens: EDTA

<table>
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<tr>
<th>Specimen Number (barcode)</th>
<th>Centre</th>
<th>Row No.</th>
<th>Col No.</th>
<th>Box No.</th>
<th>Rack No.</th>
<th>Shelf No.</th>
<th>Frzer No.</th>
<th>Usage Status</th>
<th>Date 1st frz</th>
<th>Time 1st frz</th>
<th>Date Used/Sent to others</th>
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<td>1st frz</td>
<td>11/10/2006</td>
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</tbody>
</table>
Current Knowledge of Diseases

Proteomic & Metabolomic Research

Clinical Markers

Ultrasound Markers

Known Molecular Markers

Novel Molecular Markers

Novel Sets of Molecular Markers

First-Time Mothers SCOPE Biobank

Bioinformatics

Analysis: Novel Combinations of Molecular Markers, Clinical and/or Ultrasound Predictive Tests Algorithms That Offer Accurate, Individualised Risk Scores for Each Disease
Why proteomics

- Multiple factors
- Targeted approaches failed to deliver
- Gene association studies require huge numbers
- Proteins hold the most promise for biomarker discovery
- Multiple biofluids
Protein biomarkers

- Analysis of any soluble protein material
- Advances in mass spectrometry
- Complex biological fluids
- Potential not yet realised
Pipeline in clinical proteomics

"I fed all the data in to the computer. The print-out reads 'How the hell should I know!'"
Pre-eclampsia collaboration

- Pre-eclampsia experts
  - Prof. Dr. Philip Baker
    - Professor / dean of Medicine and Dentistry, University of Alberta
    - Director of the Manchester Biomedical Research Center (UK)
  - Prof. Dr. Robyn North
    - Maternal and Fetal Medicine, King’s College London, UK
  - Dr. Jenny Myers
    - Manchester Biomedical Research Center (UK)

- Pronota
  - MASStermind®: an unbiased blood borne protein biomarker discovery platform
  - MASSterclass™: a multiplexed protein biomarker validation platform
Pronota is about making protein biomarker discovery routine

- **Discover** low abundance protein biomarkers in blood using unbiased methods
- **Verify** large number of candidates without the lengthy and costly antibody / immunoassay development
- **Deliver** best performing single markers and multiplex panels
The deep proteome: an untapped resource

Log$_{10}$ protein concentration in pg/ml in blood

1-100 pg/ml

1-1000 pg/ml

1-1000 ng/ml

1-1000 µg/ml

1-1000 ng/ml

1-1000 µg/ml

‘The actors’

Disease (class) ‘specific’ Biomarkers
5 pg/ml to 100 ng/ml

Untapped resource targeted by Pronota

‘The audience’

Non-specific Acute Phase Proteins
1 µg/ml - mg/ml levels

Exhaustively mined by existing proteomics methods

BNP Eng PSA NSE

CRP C4 C3 HSA AFP TTY α-2-M

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**What are the major differences with other LC/MS platforms?**

<table>
<thead>
<tr>
<th>Protein level</th>
<th>Peptide level</th>
<th>Quantitation</th>
<th>Separation</th>
<th>Ratio read-out</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MASStermind®</strong></td>
<td><strong>N-ter protection</strong></td>
<td><strong>Label incorporation</strong> ¹⁶O/¹⁸O</td>
<td><strong>Orthogonality based on advanced pooling scheme</strong></td>
<td><strong>Reference design</strong></td>
</tr>
<tr>
<td><strong>Of-the-shelf LC/MS</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>In MS</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>In MS/MS</strong></td>
</tr>
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</table>

- **N-ter protection**
- **N-ter selection**
- **e.g. iTRAQ**
- **Most commonly used: SCX-C18 combination**

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Pre-eclampsia biomarker program

Protein biomarker discovery
MASStermind®

- 30 samples
  - (10 PE vs. 5 Ctrls at 22 wks
  - 10 PE vs. 5 Ctrls at 26 wks)

  Various statistical / visualization tools
  Identification of (non-)differential proteins

  List of candidate biomarkers
  (63 / Pre-eclampsia project)

Biomarker assay development & validation
MASSterclass™

- Assay development
  - ~50 proteins → 11-13 weeks

- Screening of patient samples
  - 100 samples → 8-11 weeks

  Semi-absolute quantitation
  on all assays in all samples

Literature: 3 markers

Manchester

SCOPE
Discovery yielded 60+ proteins with potential - many proteins can be mapped to pathway model

**POOR PLACENTATION**

- Disturbances in IGF signaling pathways & glucose metabolism
- Oxidative stress
- Shedding of cellular debris

1. Oxidative stress

2. Disturbances in IGF signaling pathways & glucose metabolism

- Partially impaired trophoblast invasion

- Reduced blood supply

- Hypoxic placenta

- Release of placental factors & imbalance of angiogenic factors

- Exaggerated systemic inflammatory response

- Pre-eclampsia

- Immune maladaptation to paternal antigens

- Defective cell adhesion
- Defective cell migration

19

13

11
An example of ‘re-discovering’ a known marker

Endoglin

- Known to be only discriminative from week 26 → here confirmed by MASStermind® discovery

- In the single digit ng/mL range in blood
The bottleneck in early biomarker validation

- Large list of candidate biomarkers from untargeted discovery and other sources
- Restrictions of time and cost in developing large number of antibody-based assays
- High risk of making errors in candidate filtering
- Negates benefit of untargeted discovery
MASSterclass™: multiplexed targeted protein quantitation without need for antibodies

CANDIDATE BIOMARKER(S)

Internal standard:
- Isotopically labeled synthetic peptide

Recovered peptide from patient sample

Spike known concentration in patient sample

Quantitation using a mass spectrometer

Patient 1
Patient 2
Patient 3
Sample populations for the pre-eclampsia verification program – SCOPE samples

**Pre-eclampsia destined**

- 15 weeks of gestation (n=50) • Paired • 20 weeks of gestation (n=50) • Paired • Time of diagnosis (n=23)

**Pregnancy control** (complicated and uncomplicated)

- 15 weeks of gestation (n=100) • Paired • 20 weeks of gestation (n=100) • Paired • Gestational age matched (n=23)
## Population characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>PE (n=50)</th>
<th>Non PE (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>30.6 (4.6)</td>
<td>30.4 (5.4)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
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<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>76%</td>
<td>86%</td>
</tr>
<tr>
<td>Other</td>
<td>24%</td>
<td>14%</td>
</tr>
<tr>
<td><strong>Body mass index @ 15 wks</strong></td>
<td>26.6 (4.4)</td>
<td>25.4 (5.0)</td>
</tr>
<tr>
<td><strong>MAP (mmHg) @ 15 wks</strong></td>
<td>86 (8)</td>
<td>79 (8)</td>
</tr>
<tr>
<td><strong>MAP (mmHg ) @ 20 wks</strong></td>
<td>84 (7)</td>
<td>79 (7)</td>
</tr>
<tr>
<td><strong>Gestation age @ delivery</strong></td>
<td>37.3 (2.8)</td>
<td>40.1 (1.5)</td>
</tr>
<tr>
<td>&lt;34wks: n=6</td>
<td></td>
<td></td>
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<tr>
<td>&lt;37 wks: n=18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 37 wks: n=32</td>
<td></td>
<td></td>
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<tr>
<td><strong>Fetal sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>♂: 44%</td>
<td>♂: 58%</td>
<td></td>
</tr>
<tr>
<td>♀: 56%</td>
<td>♀: 42%</td>
<td></td>
</tr>
<tr>
<td><strong>Other pregnancy complications</strong></td>
<td></td>
<td>23(^{†})%</td>
</tr>
<tr>
<td><strong>Highest systolic BP</strong></td>
<td>164 (19)</td>
<td>121 (13)</td>
</tr>
<tr>
<td><strong>Highest diastolic BP</strong></td>
<td>104 (10)</td>
<td>75 (10)</td>
</tr>
<tr>
<td><strong>Multiorgan complications</strong></td>
<td>32%</td>
<td>0%</td>
</tr>
</tbody>
</table>

\(^{†}\) gestational hypertension, preterm labour, SGA, GDM
Discovery of a candidate predictive marker

Marker A, a novel marker, shows significant stand-alone performance at 15 and 20 weeks of gestation

- Marker A is quite invariant to subclasses (e.g. early onset, preterm, term)
- s-Eng prediction power is in agreement with recent report of Kusanovic & Romero et al. (Journal of Maternal-fetal and Neonatal medicine, 22, p. 1021-1038, 2009)

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>95%CI</th>
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<tbody>
<tr>
<td>20 weeks</td>
<td></td>
<td></td>
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<tr>
<td>s-Eng:</td>
<td>0.65</td>
<td>[0.57-0.73]</td>
</tr>
<tr>
<td>Marker A:</td>
<td>0.75</td>
<td>[0.67-0.83]</td>
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<tr>
<td>15 weeks</td>
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<tr>
<td>s-Eng:</td>
<td>0.61</td>
<td>[0.53-0.67]</td>
</tr>
<tr>
<td>Marker A:</td>
<td>0.69</td>
<td>[0.61-0.77]</td>
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</table>
Single markers identified with predictive potential in distinct subgroups

Marker B, a novel marker, shows stand-alone performance for predicting “term pre-eclampsia” at 20 weeks of gestation

Marker B gene polymorphism has been reported to associate with a significantly increased risk of pre-eclampsia

<table>
<thead>
<tr>
<th>20 weeks</th>
<th>AUC*</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td>Marker B</td>
<td>0.76</td>
<td>[0.68-0.82]</td>
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</table>

*Discrimination of term PE from controls, early onset PE and preterm PE
De novo prediction model building
Summary proprietary Pronota panels for pre-eclampsia prediction

### Hallmarks Pronota’s modeling efforts
- **Input**: biomarkers and easy-accessible clinical parameters
- **Focusing on strongest effects only in order to**
  1. Generate robust panels
  2. Keep number of parameters low
  3. Achieve comprehensive applicability

### Prediction

<table>
<thead>
<tr>
<th>Prediction</th>
<th>AUC</th>
<th># predictors</th>
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<tbody>
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<td>0.91</td>
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<tr>
<td>20 weeks – biomarkers only</td>
<td>0.82</td>
<td>3</td>
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<tr>
<td>15 weeks – biomarkers &amp; clinical parameters</td>
<td>0.85</td>
<td>5</td>
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<tr>
<td>15 weeks – biomarkers only</td>
<td>0.81</td>
<td>6</td>
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Hallmarks of Pronota’s pre-eclampsia prediction panel

<table>
<thead>
<tr>
<th>Sens*</th>
<th>Spec</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Ratio TP:FP</th>
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<tr>
<td>0.90</td>
<td>0.78</td>
<td>17.7</td>
<td>99.3</td>
<td>1:5.6</td>
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SCOPE Clinical risk model

<table>
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<tr>
<th>Sens*</th>
<th>Spec</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Ratio TP:FP</th>
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<tr>
<td>0.64</td>
<td>0.90</td>
<td>25.2</td>
<td>97.9</td>
<td>1:4</td>
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</table>

*Modelling based on 1000 low risk nulliparous with 5% PE
Pre-eclampsia product

A 20 week test to aid the prediction of pre-eclampsia in low risk nulliparous women, allowing appropriate stratification of care

- Additional validation ➔ populations x2
- Biomarker ELISAs ➔ under development
- Market analysis ➔ Fine tuning the product requirements
Acknowledgements

- Professor Robyn North
- Professor Philip Baker
- Robin Tuytten
- Koen Kas
- SCOPE consortium
- The Pronota team