**INTRODUCTION**

- With pre-eclampsia affecting 2-8% of all pregnancies, screening for pre-eclampsia is a focus of prenatal care.
- Current screening protocols are largely based on clinical risk factors, however these fail to accurately predict pre-eclampsia risk in 1st time pregnant women.
- An unbiased metabolomics biomarker discovery effort revealed that combinations of blood-born metabolite biomarkers have the potential to predict pre-eclampsia at about 15 weeks of gestation. (Kenny et al., 2010). These findings were based on a company focusing on the development of a metabolite based pre-eclampsia (PE) risk prediction test.

**OBJECTIVES**

- Technical fit-for-purpose testing of the prototype LC-MS pipeline.
- Independent verification of the risk stratification potential of the biomarkers through a case control study.
- Development of multivariate prediction that identify within a population of low risk 1st time pregnant women, 1. a group of pregnancies which has an increased risk of pre-eclampsia and/or 2. a group of pregnancies who has a decreased risk of pre-eclampsia.

Enrichment targets were derived from the pre-eclampsia risk in primiparous women as shown in Figure 1.

**METHODS**

**ANALYTICAL PIPELINE CONSTITUENTS**

- Dedicated EDTA plasma work-up for the extraction of metabolites, with the following key attributes:
  1. Sample fortification with a mixture of Stable Isotope Labelled (sitl) metabolites prior to work-up a) to compensate for experimental variability and b) to relatively quantify and thus compare metabolite levels across all samples.
  2. Thawout of 96 blood samples per day.
- Two dedicated LC-MS assays, with the following key attributes:
  1. Multiplex analyses of 40 metabolites and 26 SL standards distributed over 2 LC-MS assays
  2. LC: Agilent 1260 HPLC-set, Reversed Phase LC (Agilent Pursuit PFP), 10 min gradient run
  3. MS: Agilent 6460 Triple quadrupole MS equipped with Agilent Jet Stream source, 2 multiple reaction monitoring transitions per target compound
- Quality assurance protocols

**PIPELINE v1.1**

CASE CONTROL STUDY 1:

- CCS_1 was nested within the European arm of the SCOPE study (n=2456, PE = 100, non-PE = 2356).
- Samples from Cork cohort only
- 1:10 Case:Control ratio; 50 cases vs. 500 Controls
- Cases and controls * randomly selected (Non-PE, other comorbidities present)

CASE CONTROL STUDY 2 - TECHNICAL

- 150 samples
- All samples were randomized and lab personnel blinded to outcome
- Since the platform was duplicated (2 LC-MS analyses), all samples were processed twice and were assayed 2 times by LC-MS. Each of the 2 subs-studies was independently performed
- Inclusive QC samples 1500 + analyses were performed.

**ASSAYS**

- Improved robustness
- Single multiplex LC-MS analysis

**RESULTS**

**ASSESSMENT ANALYTICAL PIPELINE**

- For 95/40 targets there was more than 20% missing data within CCS_1, the data of these assays was discarded.
- Precision for the remaining 31 assays was satisfactory with 71% of the assays having a %CV ≤ 15%.
- Precision metrics are determined for the whole analytical pipeline: simple work-up, LC-MS analysis, and data processing.
- The use of SIL standards was confirmed to compensate for most of the technical variability.

**ASSESSMENT SINGLE BIOMARKER POTENTIAL**

- The predictive performance of single biomarkers was assessed by means of the Receiver Operating Characteristic (ROC). Metabolites with an area under the curve (AUC) significantly different from the null hypothesis (AUC ≥ 0.5) are reported (lower limit of the 95% Confidence Interval of the ROC-AUC ≥ 20.5).
- 731 metabolites had assigned significant predictive power for pre-eclampsia (Table 2).

**MULTIVARIATE ANALYSES**

- Application of logistic regression to predict definitive models for PE.
- Models with a maximum of up to 5 variables (metabolites and selected clinical parameters)
- Models selected based on discriminatory performance, significance of the variables
- Identify models which fulfil pre-set enrichment targets

**CONCLUSIONS**

- LC-MS was confirmed as a viable platform for multiplex analysis of metabolite biomarkers relevant to the prediction of pre-eclampsia.
- Comparing the levels of multiple metabolite markers in multivariate models enables stratification of otherwise low risk nulliparous pregnant women into high risk or low risk groups for pre-eclampsia at about 15 weeks of gestation.

**NEXT STEPS**

- Robust prediction models
- Clinical Validation of the Metabolite based test in large scale European , multicentre phase IIa clinical study
- IMPROved (Navaratnam et al. 2019)