Preeclampsia risk stratification early in pregnancy: Conversion of a promising metabolomics discovery into a LC-MS based clinical assay

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Abstract

Basic metabolomics research has uncovered that combinations of blood borne metabolites can risk-stratify women early in pregnancy according to their risk of developing preeclampsia later in their pregnancy. Since then, a company has been established which is dedicated to translating this finding into a tool for health care providers and pregnant women. A targeted approach is being developed whereby ca. 40 metabolites are (semi-) quantified using liquid chromatography-tandem mass spectrometry. An update on the method development progress as well as an overview of the clinical studies lined-up to verify and validate the preeclampsia risk stratification test will be discussed.

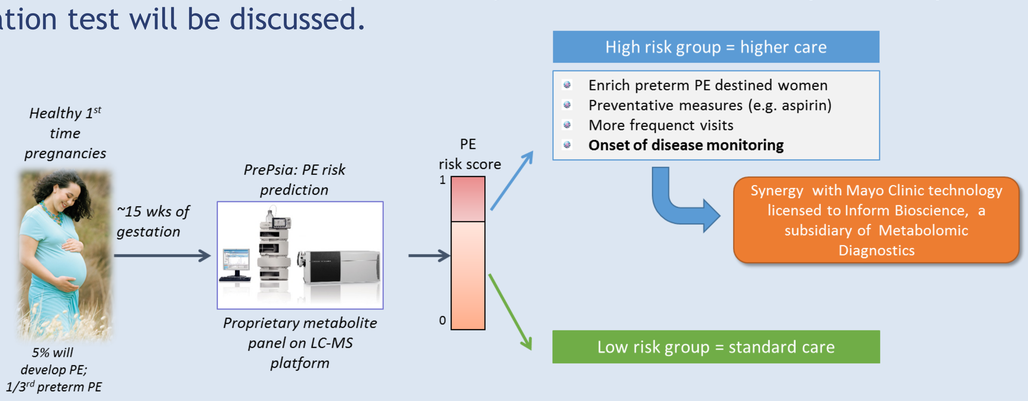


Fig.1. Overview Metabolomic Diagnostics' Preeclampsia Risk Stratification product

Background

- Preeclampsia is mostly a syndrome of late pregnancy characterised by concomitant (new onset of) hypertension (high blood pressure) accompanied by new onset proteinuria (elevated protein in the urine) or signs of multi-systemic impairment. Currently there is no cure for preeclampsia other than delivery.
- Preeclampsia has been the most significant cause of maternal death over recent decades: between 70,000 and 80,000 women die every year from preeclampsia and in excess of half a million new born infants die annually as a direct result of the condition.
- Efficient prediction of preeclampsia is considered a crucial step stone to deliver more effective prenatal care, minimize preeclampsia related complications and per result reduce health care costs.

The Challenge

- Thus far preeclampsia prediction is largely depending on clinical risk factors, but these are marginally useful in healthy first time pregnant women who account for >50% of preeclampsia cases.
- Prediction of preeclampsia in first time pregnant women requires a panel of biomarkers in order to encapsulate the complex pathogenesis of the syndrome.
- Research and (commercial) test development requires prospectively collected 1st pregnancy biobanks, whereby 1000's of women need to be longitudinally monitored and sampled throughout pregnancy.

The Opportunity

- Kenny *et al*[1] from the University College Cork, Ireland found that accurate prediction of preeclampsia in 1st time pregnant women is possible using a panel of blood-borne metabolite present in plasma of ~15 weeks pregnant women.
- In recent years there is an increased demand for the rationalisation of health-care: identification of 1st time women at risk of preeclampsia will allow health-care workers to better administer the right prenatal care to the right women.
- Metabolite analysis using LC-MS is well established in pharma (bioanalysis) and neonatal screening ("heel prick"). LC-MS is therefore an obvious platform choice to port a potentially disruptive preeclampsia risk stratification test into the clinical laboratory.
- Together the above present an entrepreneurial opportunity to develop a disruptive, first-in-class diagnostic tool to stratify 1st time pregnant women early in pregnancy according to their preeclampsia risk.

The Approach

- Collaboration between Company, technology inventors and other clinical experts.
- Creation of Public-Private partnerships to lever transnational funding to propel both academic research and SME-based product development.
- Seminal partnership with dedicated instrument manufacturer.
- Understanding of funding mechanisms – what funding to apply / seek at what stage of company / product development.

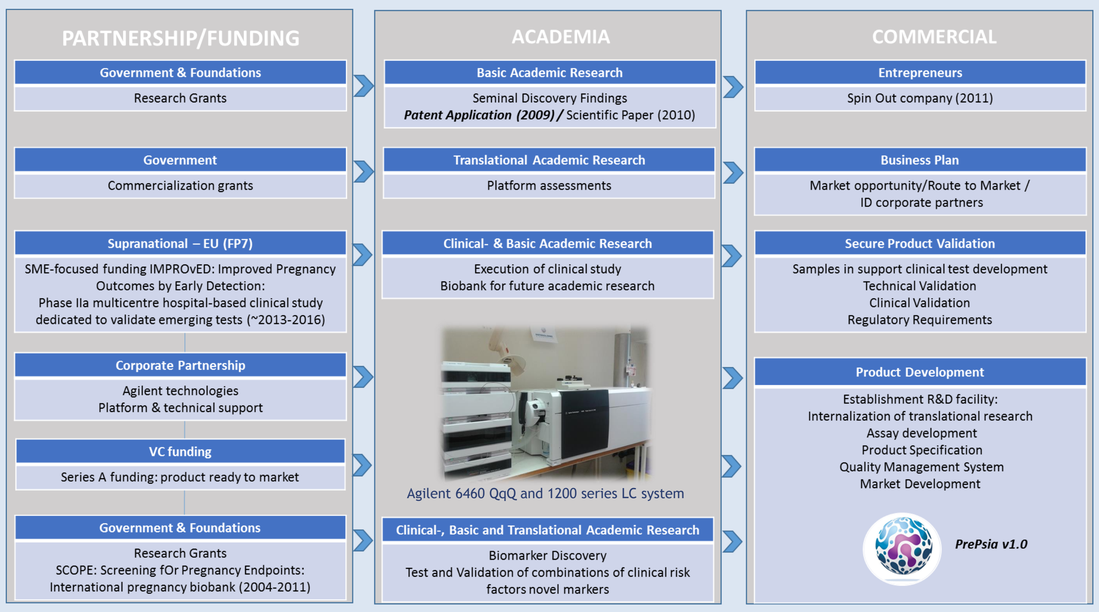


Fig.2.Overview of the interplay between funding bodies, academia and company

Method Development

Selection of Target Metabolites

- Primarily based on metabolites identified in Kenny *et al*. (2010)[1]; 41 Target Metabolites selected
- Selection of corresponding stable isotopically labelled standards chosen based on the metabolite classes; 16 ISTDs selected

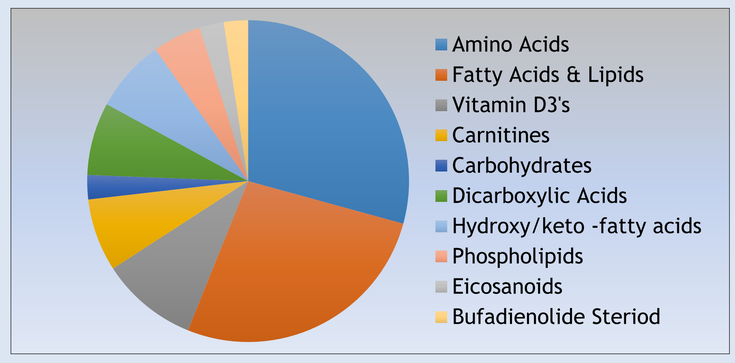
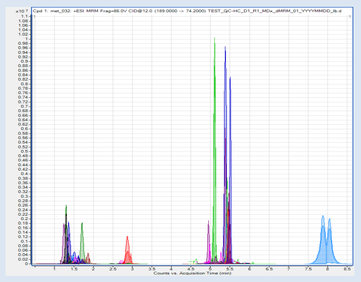


Fig.3. Composition of metabolite panel

Development of multiplex LC-MRM assay for target metabolites and selection of ISTDs



LC:
10 minute gradient elution –Agilent PFP Pursuit col; separation of hydrophilic and hydrophobic compounds

MS:
Unique Qual and Quant transitions for 41 Target Metabolites and 16 ISTDs: 112 MRM transitions / run (dMRM)

Development of a simple, comprehensive plasma extraction procedure

- Metabolite extraction procedure from plasma developed and optimised for all metabolites (and ISTDs) and compatible with LC-MS analysis methodology.

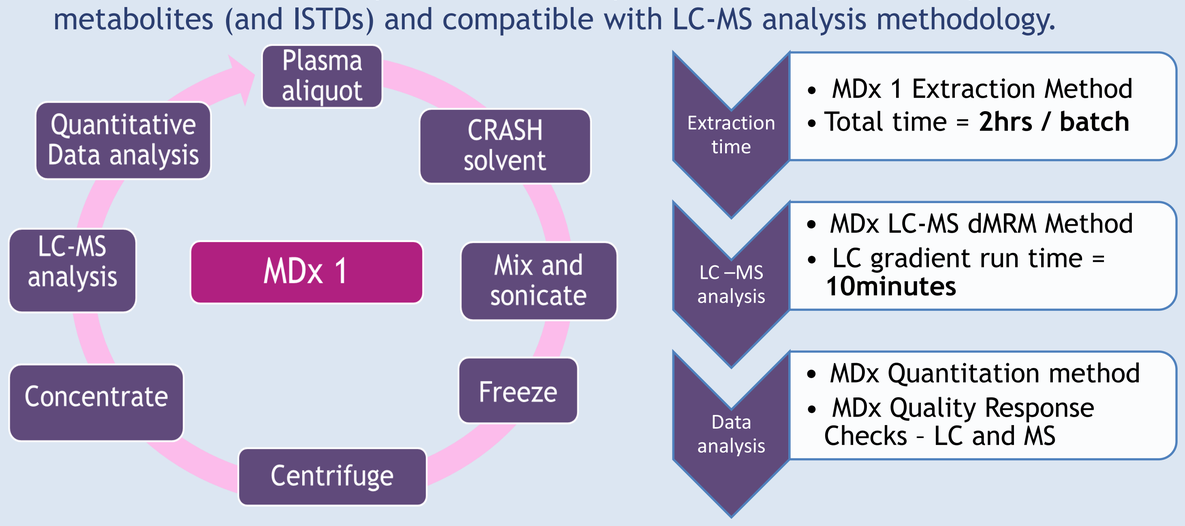


Fig.4. Metabolite sample processing pipeline

Proof of Principle: Determination of target metabolites in pregnancy blood

- 41 metabolites of interest are assay-able in pregnancy blood (15wks) with good precision
- **Semi quantitative data** generated using Relative ISTD quantitation
- Current throughput: 435 samples/ week (QC samples excluded)
- **Ongoing**: stability testing batch processing and reference range determinations

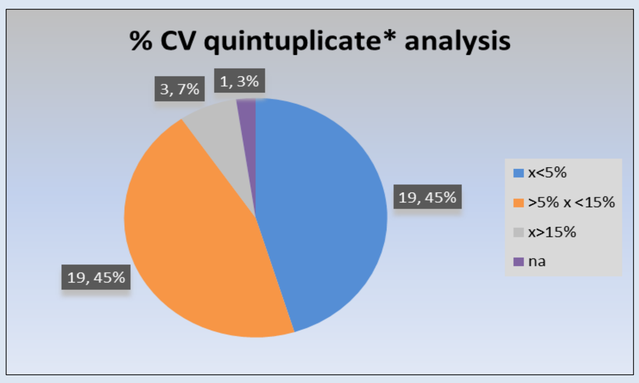


Fig.5. Precision metrics 40 metabolite assays; * 5 independently prepared samples - %CV for whole sample processing pipeline

The Near Future

- Case Control study – SCOPE (n ~700): Metabolite candidate confirmation and prediction algorithm refinement.
- Final Standard Operation Procedures for processing pipeline and data analysis.
- Field testing of SOPs and verification of algorithm in full SCOPE Europe (n ~2500).
- Technical and clinical validation of PrepSia v1.0 in IMPROvED (n ~5000)[2,3].

Conclusions

- Within Metabolomic Diagnostics, a single step metabolite extraction and a targeted LC-QqQ-MS approach using stable isotope labelled metabolites for relative quantification has been successfully developed.
- All major components are in place to commence processing the clinical samples as available in SCOPE and IMPROvED biobanks.

References

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