Proteomic Discovery of Serum Biomarkers Predictive of Preterm Birth

JJ Boniface, TC Fleischer, CL Bradford, JS Flick, AD Gassman, I Ichtovkin,T Pugmire, RD Severinsen, AC Fox, S Rust, AJ Lueth, AD Poliptia, GC Critchfield and GE Hickok

Sera Prognostics Inc., Salt Lake City, UT

www.seraldagnostic.com

Abstract

Introduction

The etiologies of preterm birth (PTB) include factors such as infection, placental hemorrhage and stress. Existing tests have poor performance and are limited in utility, especially in high-risk patients. Thus, there is a compelling need to identify biomarkers of PTB to enable interventions for patients at risk.

Methods

The Proteomic Assessment of Preterm Risk Clinical Trial (11 sites) utilized infants born at term as controls for the 67 possible infants enrolled in 2 phase discovery strategy (Fig. 1). First, candidate protein biomarkers were ascertained by both hypothesis dependent databases queries and first pass shotgun proteomics discovery effort. Second, a single MRM-MS essay was developed to quantify over 220 candidate biomarkers in serum samples from 109 controls and 180 cases. Ultrasensitive and LC/MS/MS workflows for protein identification were assessed by a booth method with clinical and laboratory based models.

Results

Univariate analysis of the MRM-MS discovery studies identified analytes amongst multiple pathways and functional categories of relevance to PTB. The resulting discovery panel was selected using both supervised and unsupervised models and evaluated further.

Hypothesis Dependent Curation

Key word searches were conducted using publicly available databases. Keywords included relevant clinical terms (e.g. preterm birth), their synonyms and related terms in maternal medical practice (e.g. pre-eclampsia, intraventricular hemorrhage, etc.). Clinical search terms were combined with terms such as “biomarker”, “prognostic”, “predictive”, “blood”, “serum”, “plasma”, etc.

Candidate proteins were extracted and mapped to the best RefSeq and Uniprot identifiers. When possible relevant splice variants were identified.

Candidate proteotypic tryptic peptides and their corresponding transitions were selected using proprietary and public databases.

Hypothesis Independent Discovery

Process workflow

Serum samples were depleted of the 16 highest abundance proteins using the Agilent Human 16 Multiple Affinity Removal Column (MARS-16), digested with trypsin and subjected to MudPIT analysis using LTQ Orbitrap.

Univariate analysis was used to elect proteins for incorporation into the MRM-MS assay.

Graph showing reproducibility of MARS-16 depletion.

Univariate classification results.

Process Performance

Graph showing that transitions to the same peptide and peptide to the same protein were highly correlated, indicative of a robust assay.

Conclusions

Out 2-phase strategy lead to the identification of biomarkers across multiple pathways of relevance to PTB.

Good performance in training sets using multivariate models constructed from these analyses suggests that together they are promising candidates for prediction of PTB.

Once classifiers have been finalised, their performance will be assessed in follow-up validation studies.

References


Joy Boniface
jonfleischer@seraagnostics.com