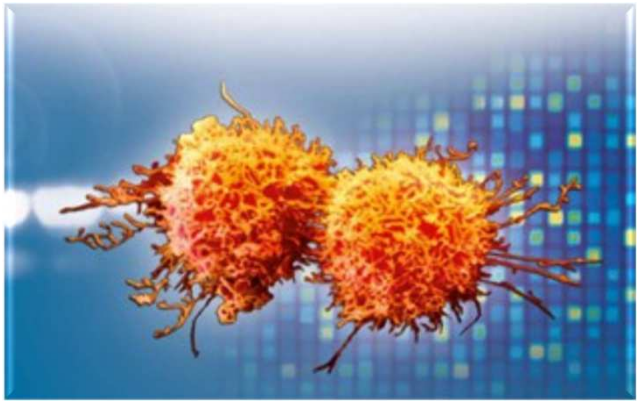
Molecular Diagnostics Symposium

28 February & 1 March 2019, Zurich



Noninvasive blood tests for fetal development predict gestational age and preterm delivery

Stephen R. Quake

Science **360**, 1133–1136 (2018)

Bernard Conrad

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Médecine génomique
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MEDICINE

Noninvasive blood tests for fetal development predict gestational age and preterm delivery

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Determination of gestational age

- Estimate of last menstrual period: ±precise
- Estimated date of delivery: +9 calendar months + 7 days (280 d) to first day of last menstrual period
- Accuracy: accurate recall of mother, regular 28 d cycles, conception day 14 of cycle
- +/- 7 days of estimated date in 49.5%*

- Ultrasound: more accurate but expensive
- Ultrasound uses fetal size to determine gestational age (time since first day of last menstrual period)
- Accuracy: varies according to the gestational age, in 1st trimester most accurate
- +/- 7 days of estimated date in 55.2%*

«Knowledge of day of ovulation in a cycle, in which conception occurs, if available, appears from this study to provide the most reliable estimate of gestational age»†

*Am J Obstet Gynecol 1996; 174:278-81; †Arch Gynecol Obstet 2016; 294:867-876

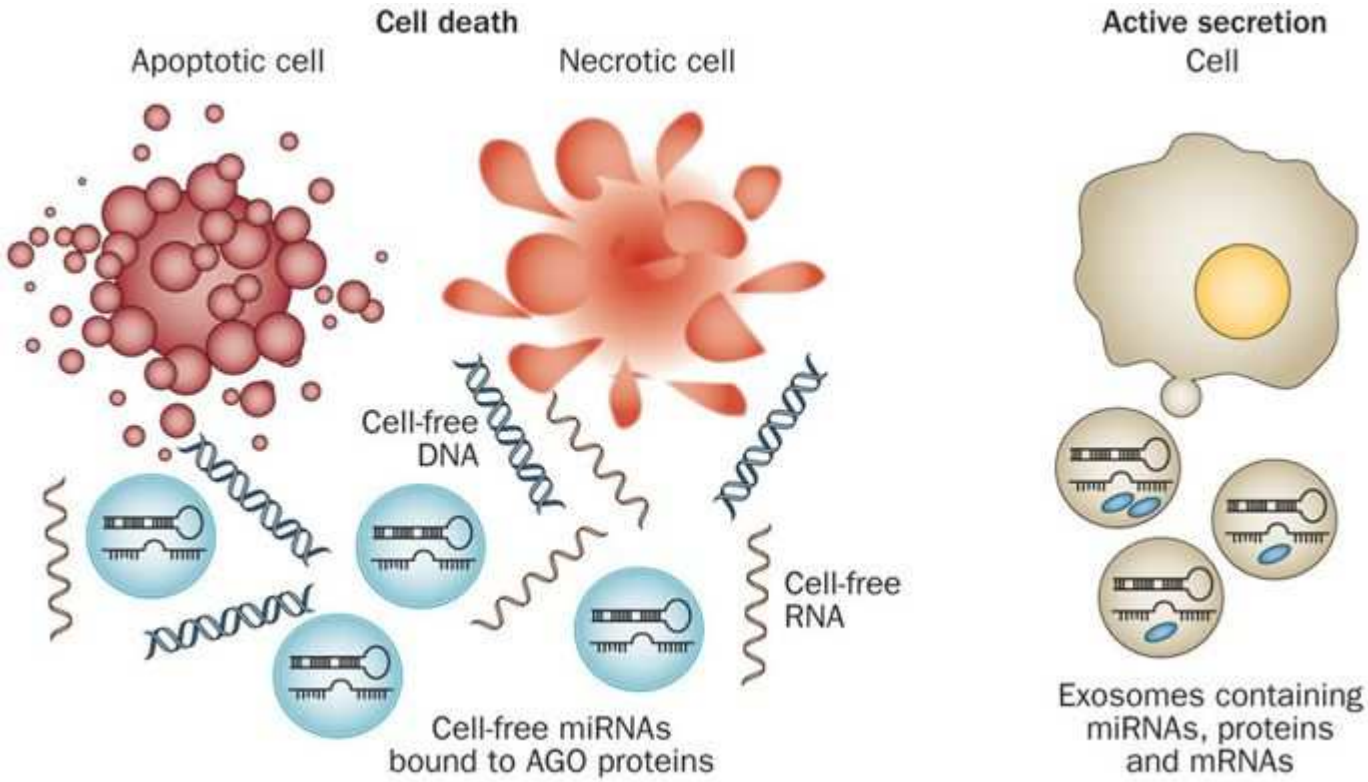
Estimates of delivery do not account for premature birth

Premature birth

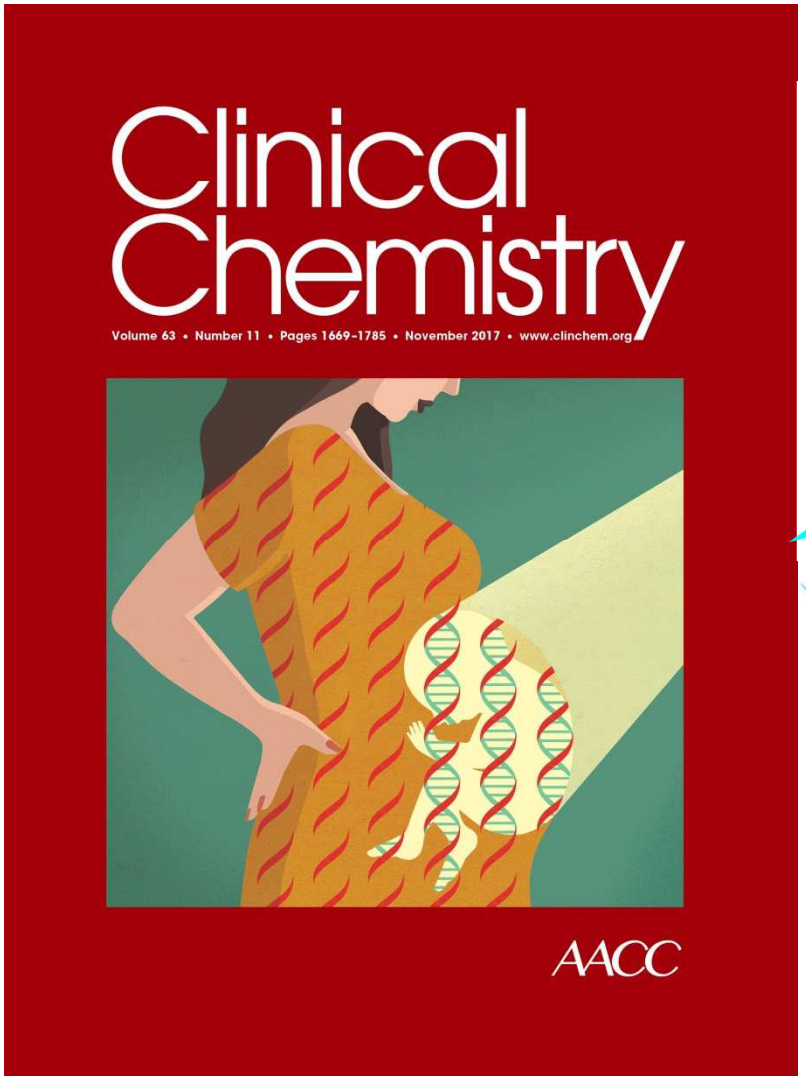
- 15 million neonates worldwide, 5-18% of births, 60% in low and middle income countries
- leading cause of neonatal mortality & later morbidity
- two thirds spontaneous → identify pregnancies at risk

BMC Pregnancy Childbirth 2014; 14:56

Cell-free nucleic acids & cfRNA



Nature Rev Clin Oncol 2014; 11:145-156



Plasma cfRNA signature to predict **gestational age**?

Plasma cfRNA signature to predict **preterm delivery**?

Strategy & outcome

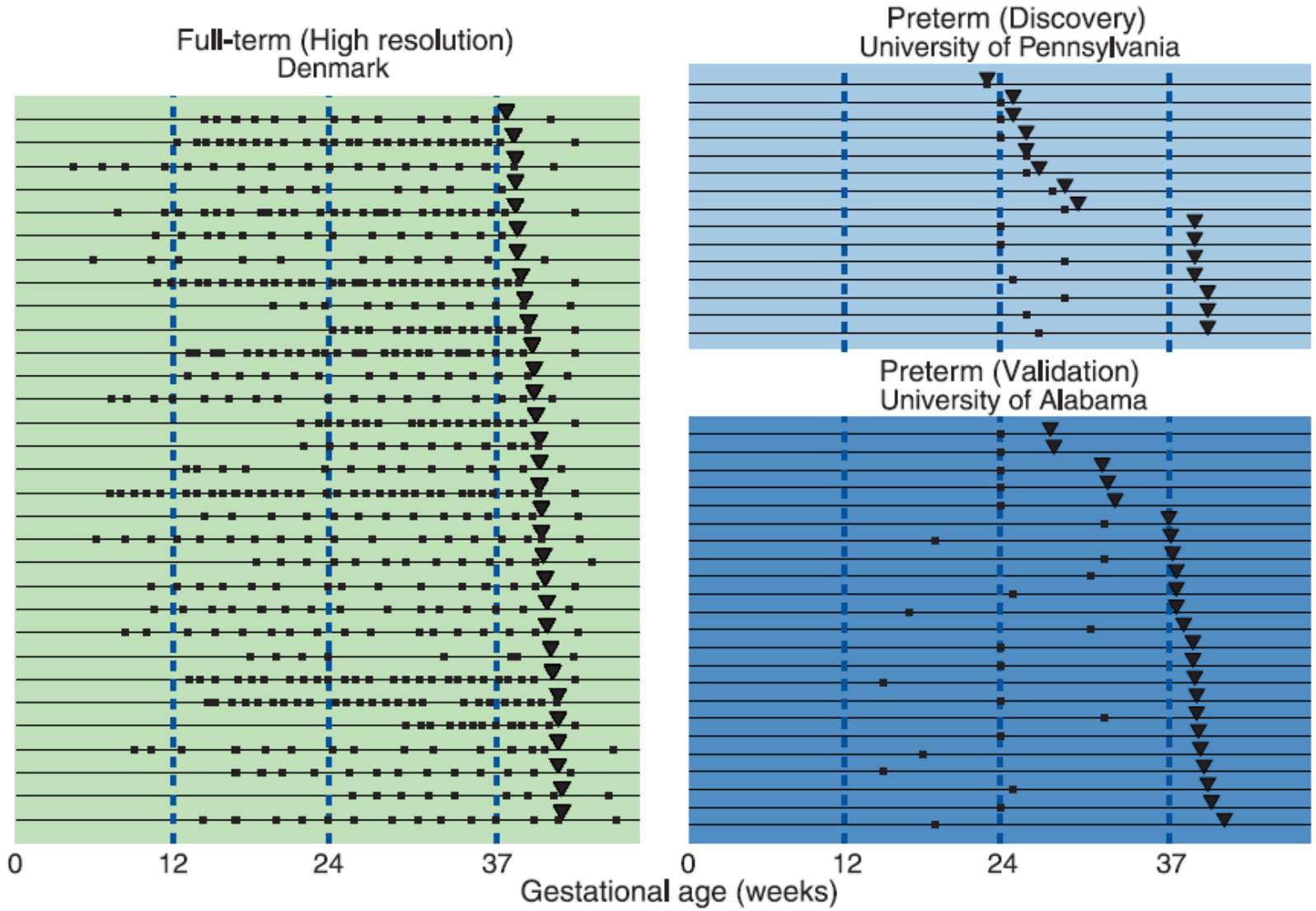
Determination of gestational age (GA)

- Full-term pregnancy samples analyzed by RT-qPCR using placenta- and immune system-specific genes, as well as fetal liver-enriched genes
- Machine learning: 51 gene- and **minimal placenta-specific 9 gene signature determining GA** "similarly" to US

Determination of preterm delivery

- Preterm samples analyzed by RNA-Seq & RT-qPCR
- Significance tests for differentiating genes & clustering: 38 gene signature capable of separating full-term from preterm
- **Classifier with top 7 cfRNAs** : correctly classifies 6/8 preterm samples misclassified 3/18 full-term samples

Three sample cohorts for full-term (DK) and full-term/preterm pregnancies (Penn/Ala)

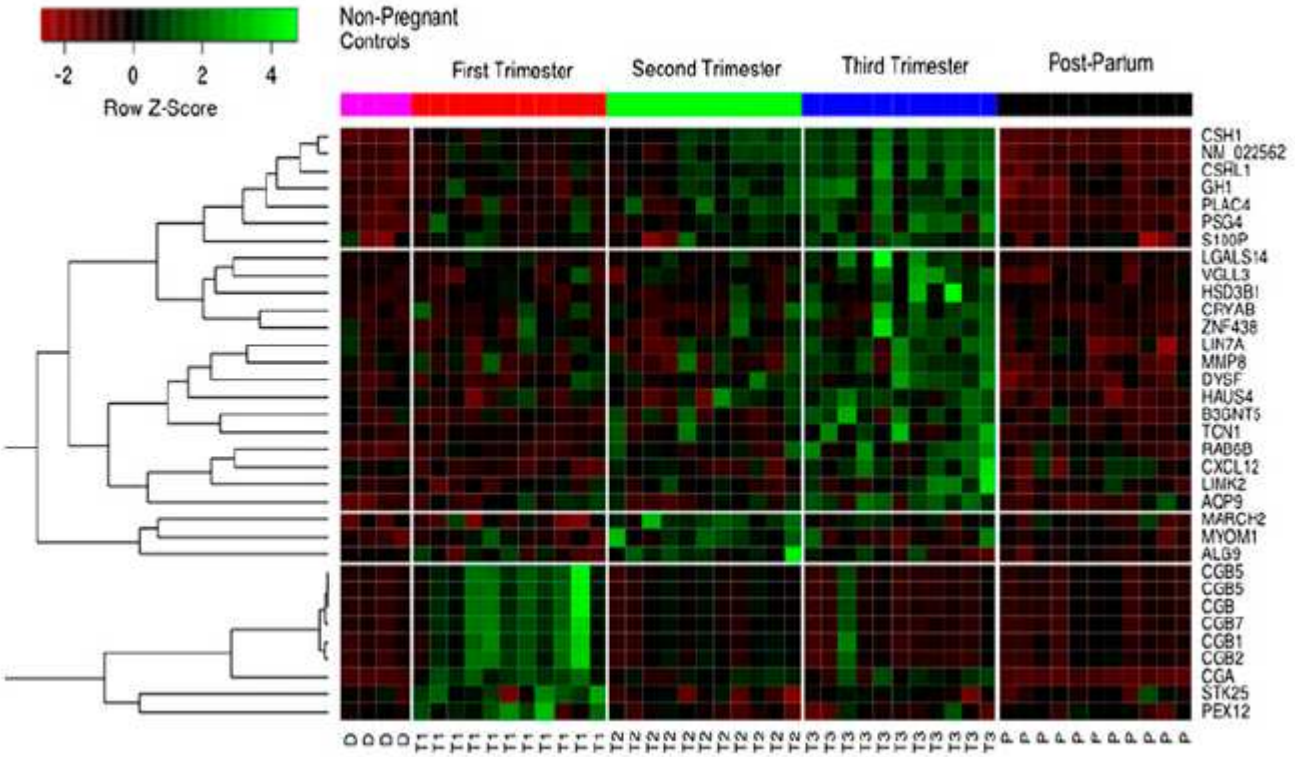


Fetal cfRNA profiling during pregnancy

Heatmap of time-varying genes identified from microarray analysis.

T1<T2<T3

T1



Chorionic gonadotropin (CG) family of transcripts

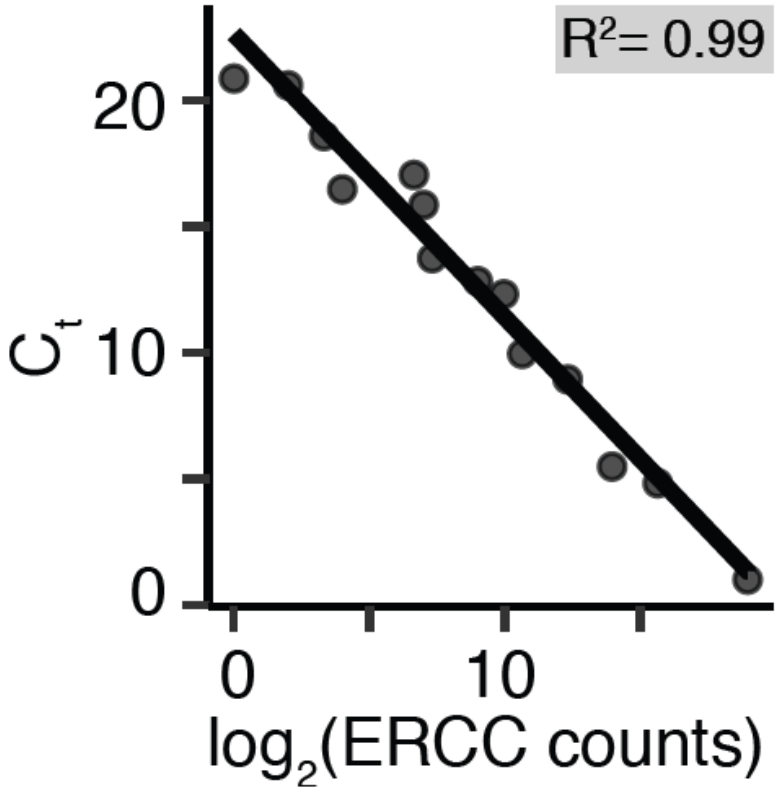
- Produced by placental syncytiotrophoblast
- βHCG to detect pregnancy

Time course with 15 samples

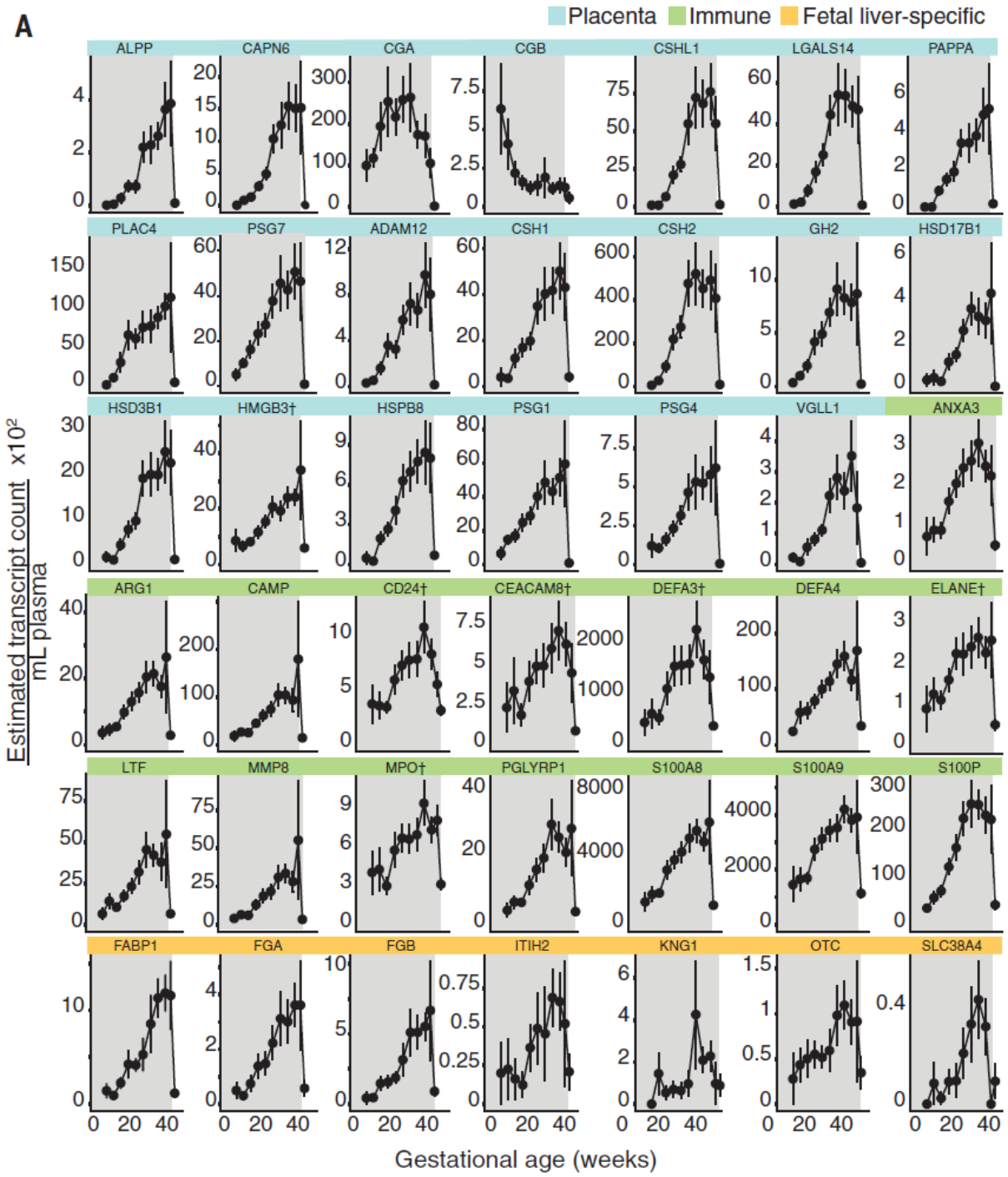
- 11 pregnancy & postpartum
- 4 non pregnant

PNAS | May 20, 2014 | vol. 111 | no. 20 | 7361–7366

cfRNA measurements with highly multiplexed RT-qPCR (microfluidics)



Linear regression for threshold cycle (C_t) versus External RNA Controls Consortium (ERCC) RNA control shows that measured values agree with linear fit (R-squared=0.99).

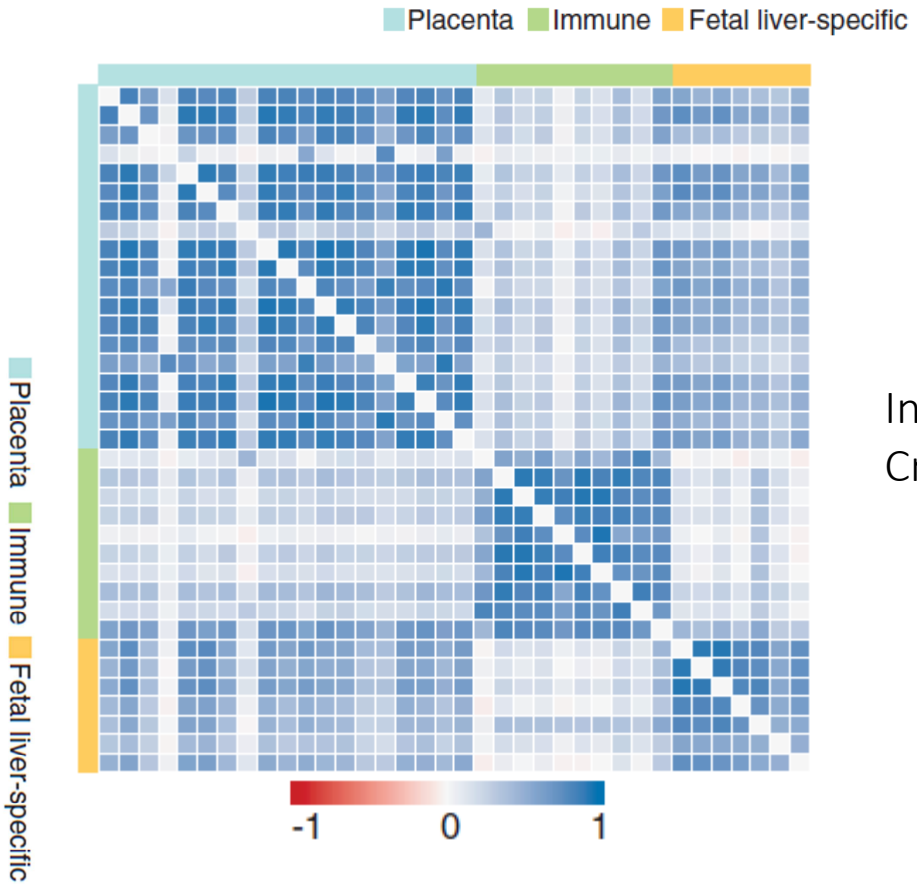


Mean ± SEM cfRNA

- 21 women†
- 31 women

Placental- immune- and fetal liver-specific gene-pairs are correlated

Heat map of Pearson correlation coefficient for each gene pair

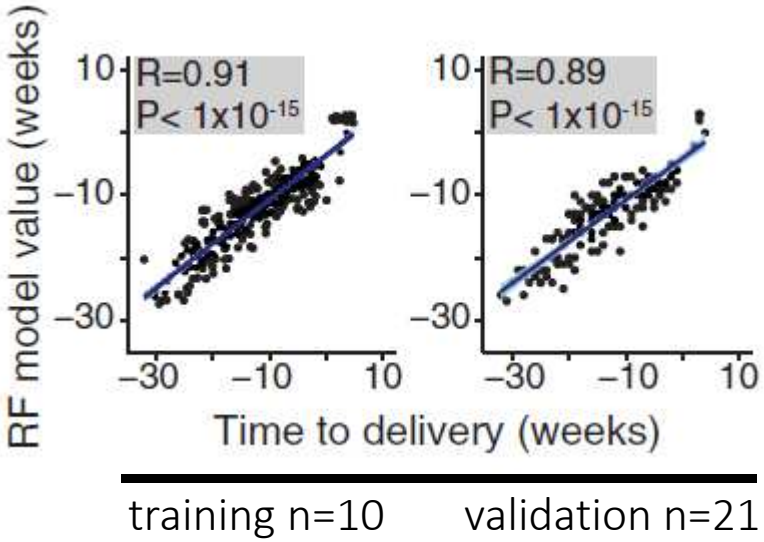


Inter-correlation ≈0.8

Cross-correlation ≈0.5

Cross-validated random forest model predicts time to delivery

= 100% of samples will appear in training & validation set, using 10 partitions



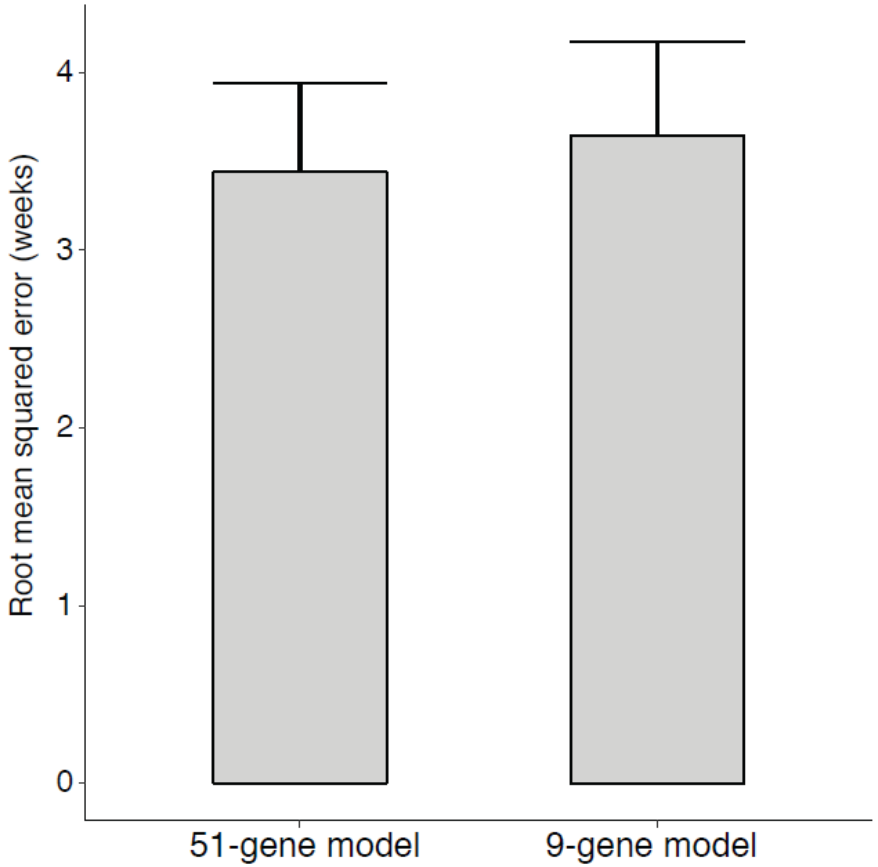
training n=10 validation n=21

DK data set

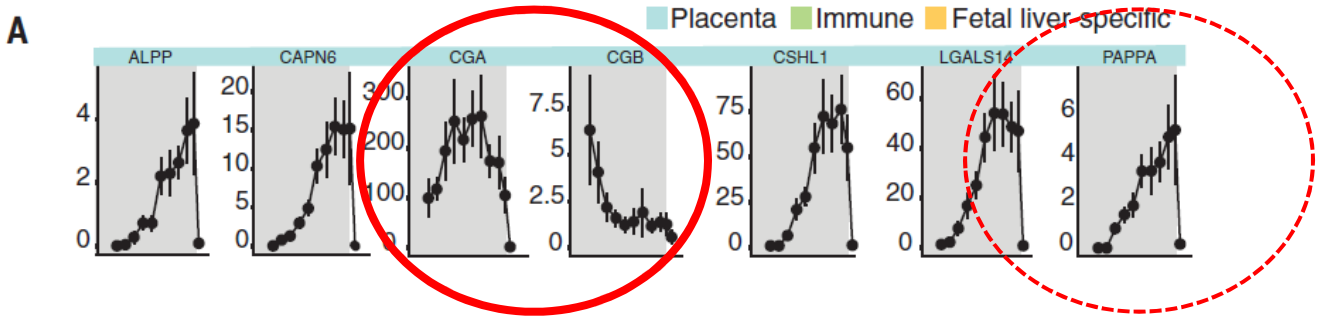
- cfRNA to predict time from sample collection until delivery
- Using R in caret package (Max Kuhn, J Stats Software 2008; 28:1-26)

A. Criminisi, J. Shotton and E. Konukoglu, Microsoft Research technical report TR-2011-114

9 placenta-specific cfRNA equivalent predictive power than full 51 gene panel



Key features of the model



- *CGA* + *CGB*: α- and β3-subunits of HCG: pregnancy biomarker
- *PAPPA*: pregnancy-associated plasma protein A associated with pregnancy risks such as aneuploidy and preterm birth*

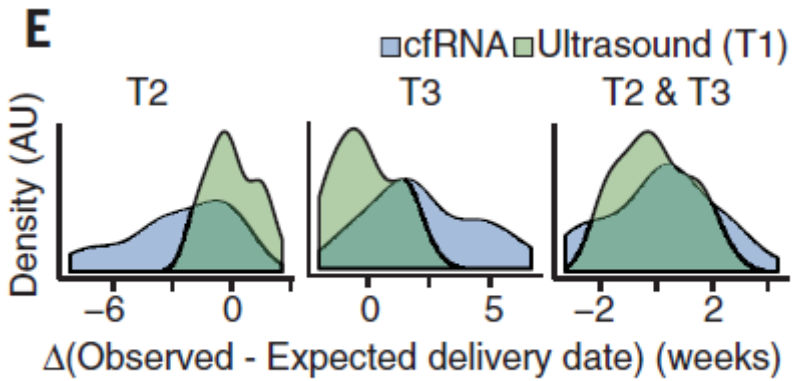
*Ultrasound Obstet Gynecol 2008; 31:147-52

Model versus established tools to predict GA

Table 1. Comparison of gestational age estimates using cfRNA and ultrasound. Distribution of difference between estimates of gestational age, which assume delivery at 40 weeks gestation, and observed gestational age at delivery listed for four distinct methods, where *n* indicates the number of women included. Gestational age was estimated using cfRNA measurements from the second (T2), third (T3), or both (T2 and T3) trimesters and ultrasound measurements from the first trimester (T1).

Method	Δ [observed − expected delivery date (weeks)] (%)				
	< −2	−1 to −2	±1	+1 to +2	> +2
cfRNA (T2, *n* = 28)	50	18	32	0	0
cfRNA (T3, *n* = 31)	0	6	23	29	42
cfRNA (T2 and T3, *n* = 31)	19	6	45	10	20
Ultrasound (T1, *n* = 31)	0	26	48	23	3

Model versus established tools to predict GA



II

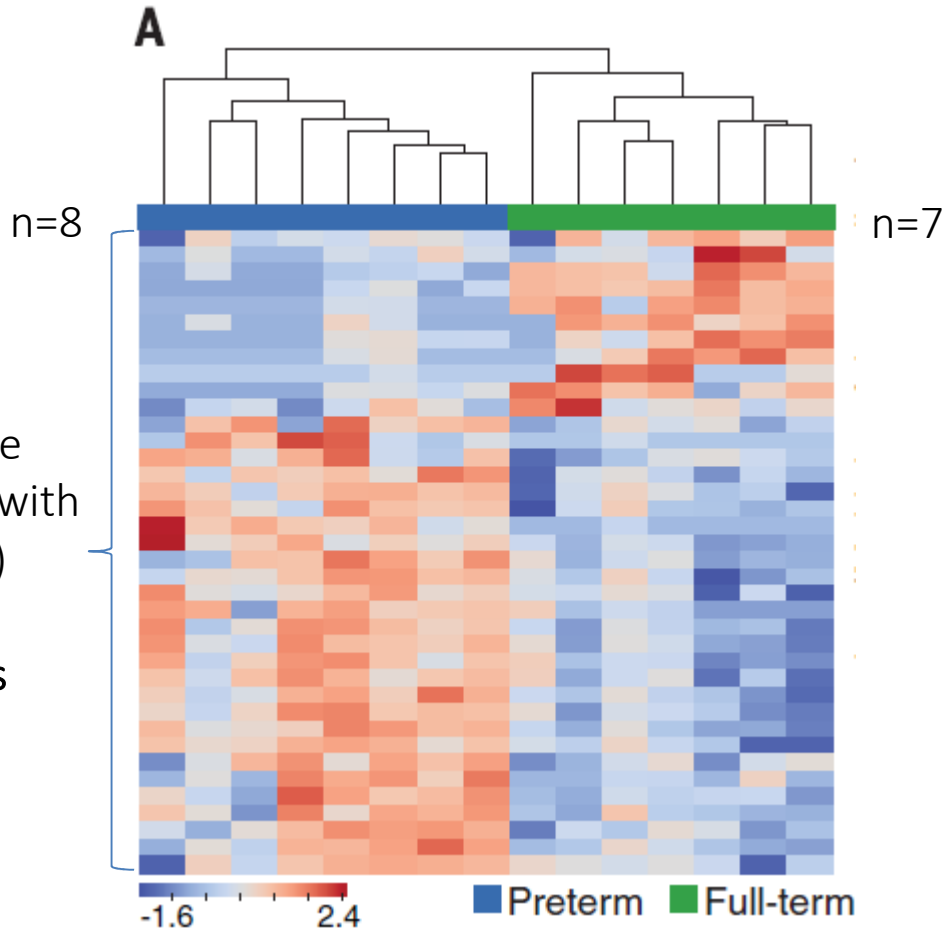
cfRNA signature for preterm versus full-term pregnancies

Heat map for 38 differentially expressed genes in preterm vs full-term pregnancies
Preterm biomarker candidate discovery using cfRNA-seq

cfRNA-Seq in Penn data set

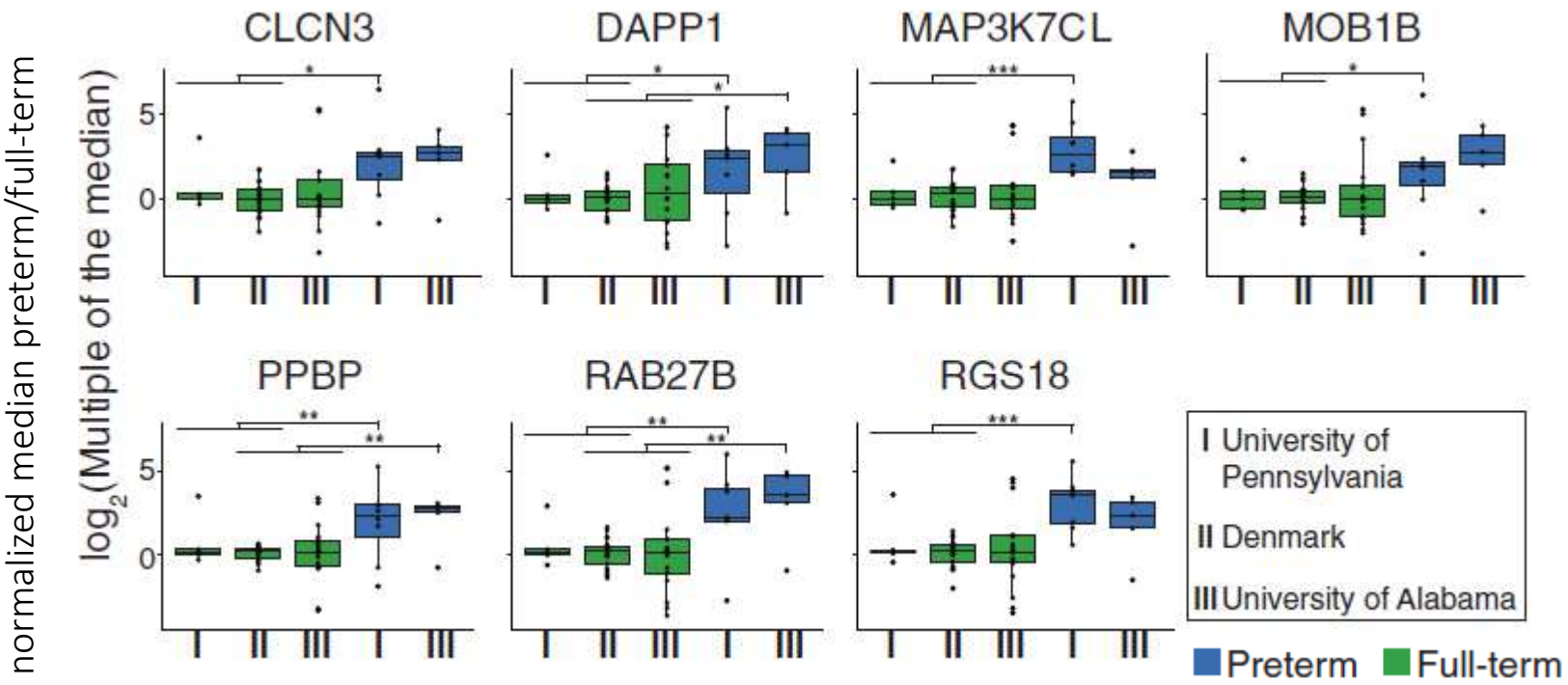
- NextSeq 10 million reads/sample
- Differentiating genes identified with three significance tests (edge R)

- **38 differentially expressed genes** separate full-term vs preterm $P < 0.001$



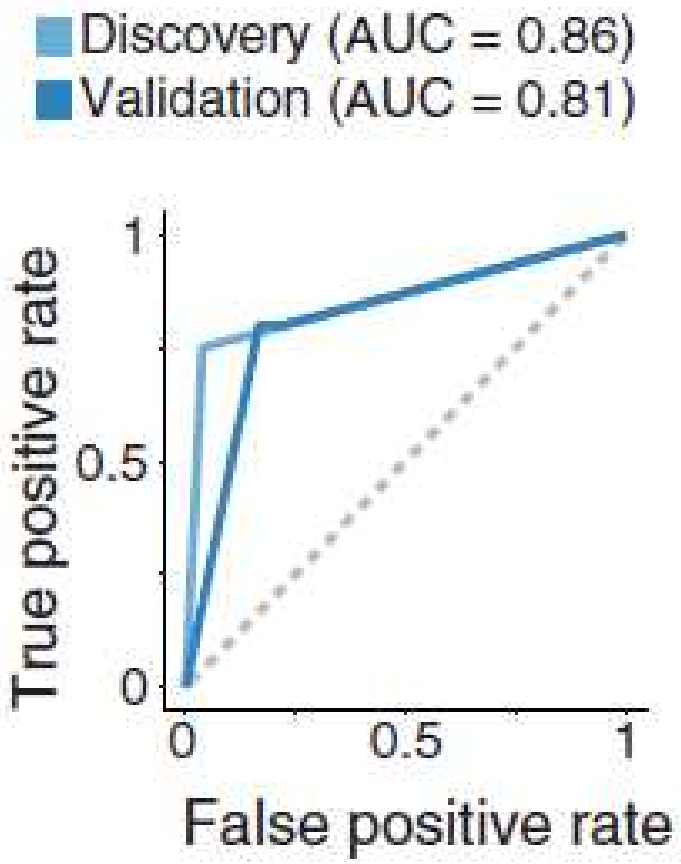
Classifier with top seven cfRNA

Preterm biomarker candidate discovery using RT-qPCR

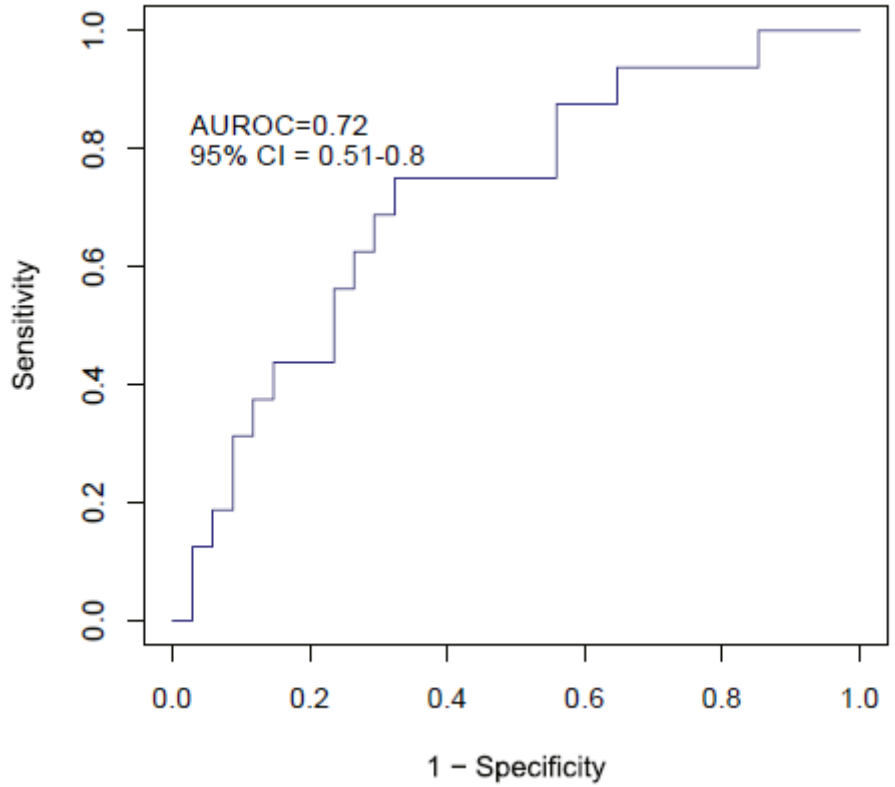


- Mean ± SD of differentially expressed genes validated using qRT-PCR
- Separate data with multiples of median cutoff 2.5
- accurate classification of 4/5 preterm pregnancies [80%]
- misclassified 3/18 full term samples [17%]

Receiver operating characteristics for classifier to separate preterm from full-term pregnanices



cfRNA versus other biomarkers: MS-determined log ratio IBP4/SHBG

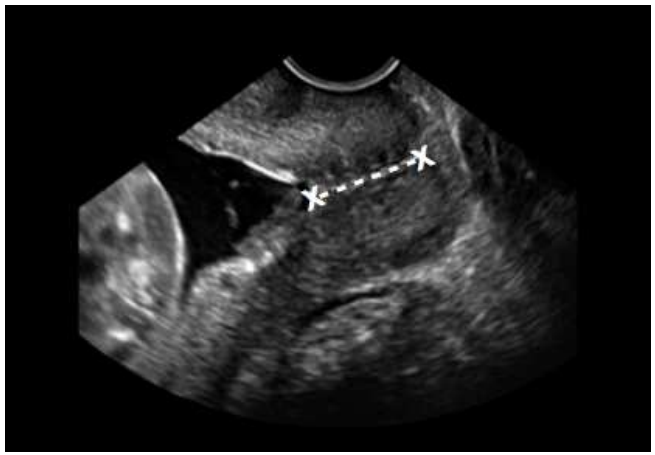


- MS: insulin-like growth factor-binding protein 4 IBP4 + sex hormone-binding globulin SHBG

Am J Obstet Gynecol 2016; 214:633.e1-633.e24

cfRNA versus fFN & CL

- Cervical/vaginal fFN (fetal fibronectin)
- Cervical length determined by US
- In combination sensitivity and specificity were 75% and 71%
- Cervical length (CL) > 30mm and present cervical gland area 96-97% negative predictive value for preterm delivery



J Pregnancy 2012;2012:201628

Conclusions

- Plasma cfRNA signatures provide potentially promising biomarkers for routine clinical diagnostics = solid science
- Randomized study, larger sample size and diverse ethnicities required
- Preterm cohorts biased by elevated pre-test risks
- AUC profile slightly more favorable than competing biomarkers for determining of birth at risk for preterm
- GA determination potentially useful alternative to US

Why didn't we and will we not adopt these new biomarkers?

- "Potential Excessive Testing at Scale
  Biomarkers, Genomics, and Machine Learning"*
- Many new biomarkers emerging that are only marginally better than existing ones, and that will lead to more testing but not necessarily to better healthcare
- "important to ensure a system that benefits patients and improves their health"*

**JAMA.* 2019;321(8):739-740.doi:10.1001/jama.2019.0286


Thank-you