

# **Using cell-free RNA to predict gestational age and preterm delivery**

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# Current tests that estimate gestational age and delivery date are inadequate

What is the fetus' gestational age?

## Molecular tests

Cannot predict gestational age

## Ultrasound imaging

Expensive

Best done in 1<sup>st</sup> term

## Last menstrual period

Unreliable

When will a given pregnancy deliver?

## Molecular tests

Limited BMI range

Limited gestational age range (2 weeks)

Low positive predictive value

## Ultrasound imaging

Expensive

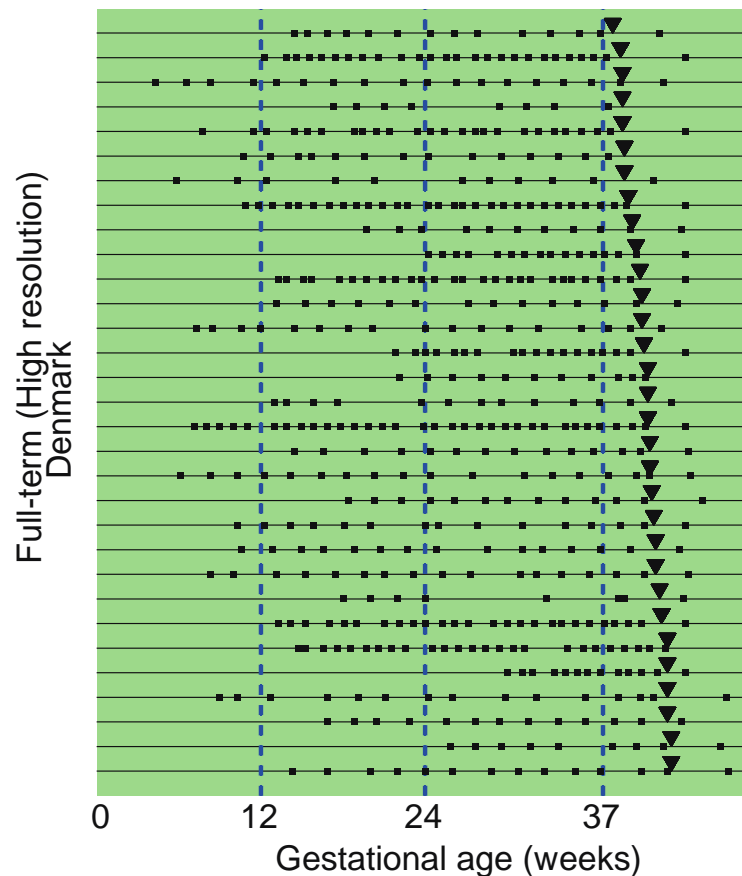
Low positive predictive value and specificity

## Demographic factors

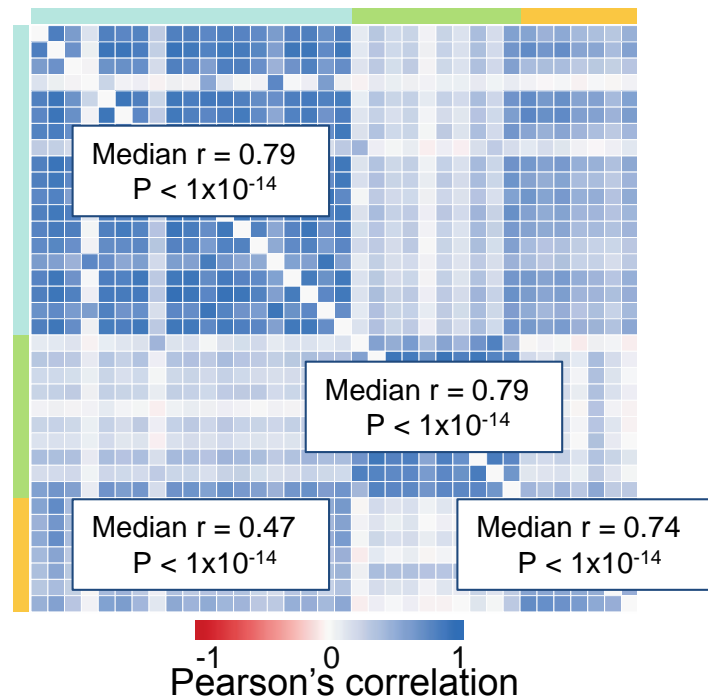
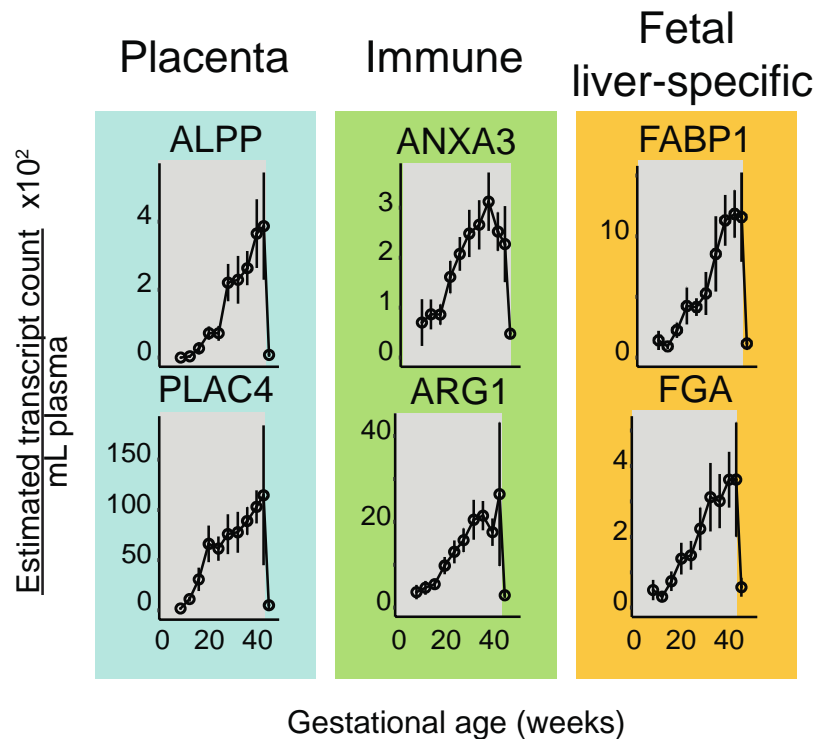
Unreliable

**Developing an accurate and  
inexpensive non-invasive test to  
predict gestational age**

# Studying full-term pregnancy at high time-resolution



# Time course of gene expression differs by gene function



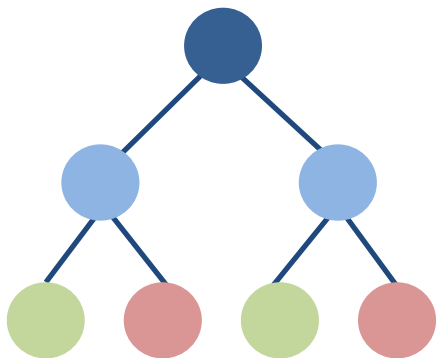
**Can we leverage these changes over time to predict gestational age?**

# Applying machine learning to predict gestational age

## Building a random forest model:

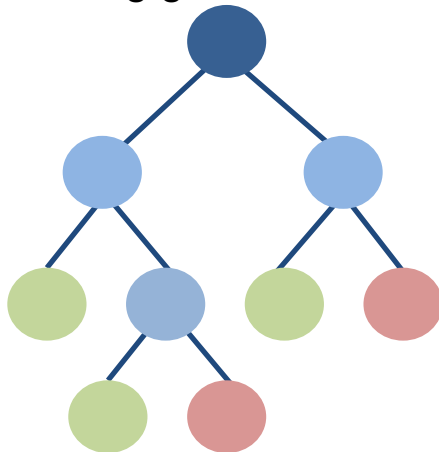
**Tree 1**

Built using genes 1-5



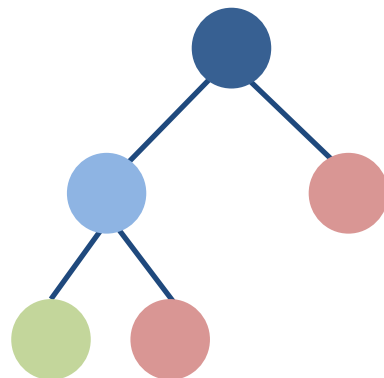
**Tree 2**

Built using genes 3-5, 10, 13



**...Tree n**

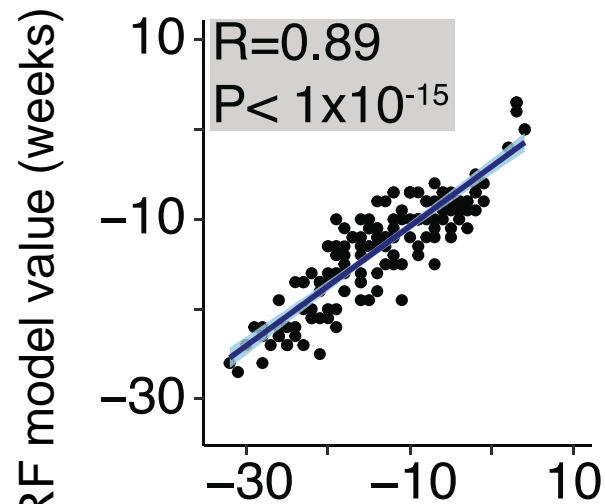
Built using genes d-g



**The mean across the trees yields prediction**

# Random forest model built using cfRNA measurements predicts time to delivery

Validation set (n=10)

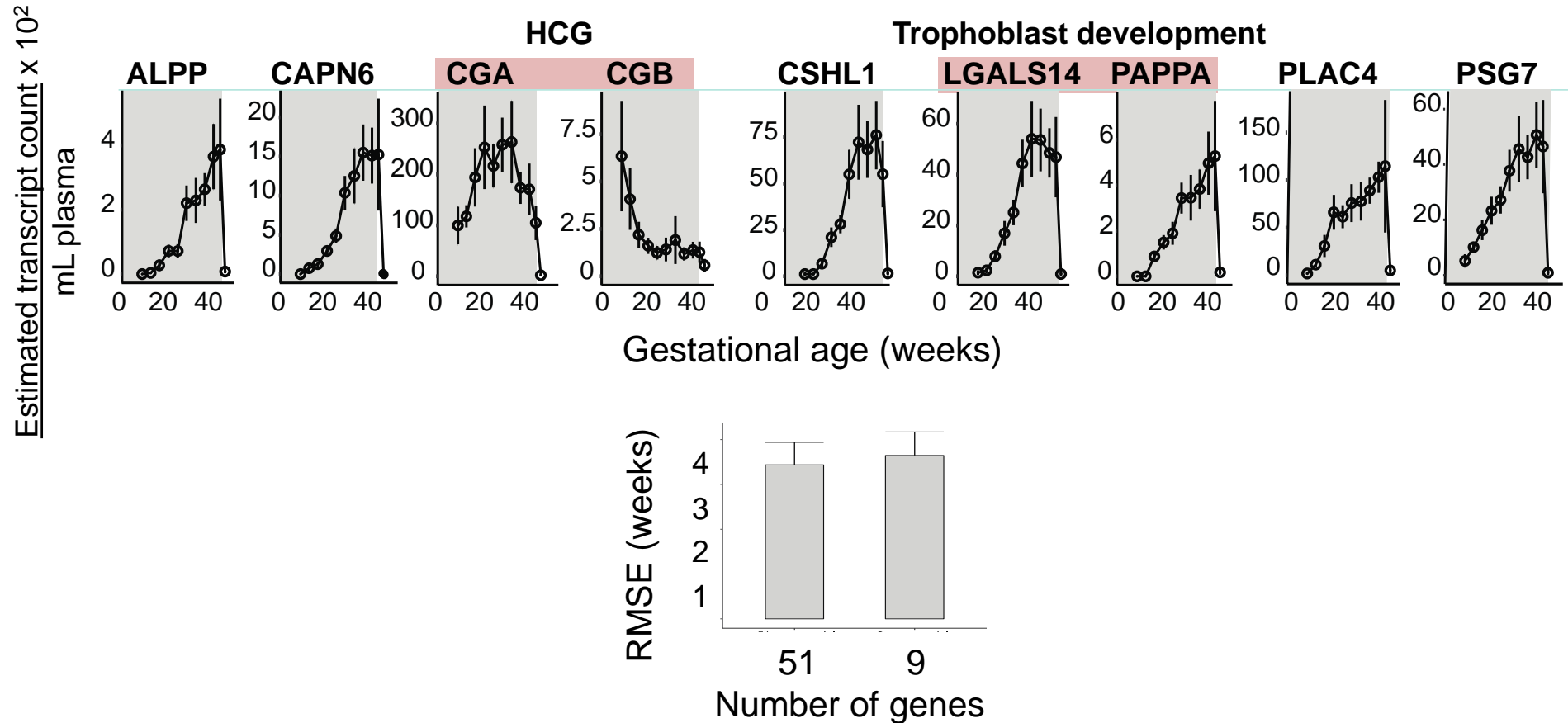


Root mean squared error (RMSE)

Term	RMSE (weeks)
T1 (<12 weeks)	5.4
T2 (12-24 weeks)	4.2
T3 (>24 weeks)	3.8
Post-partum	2.6

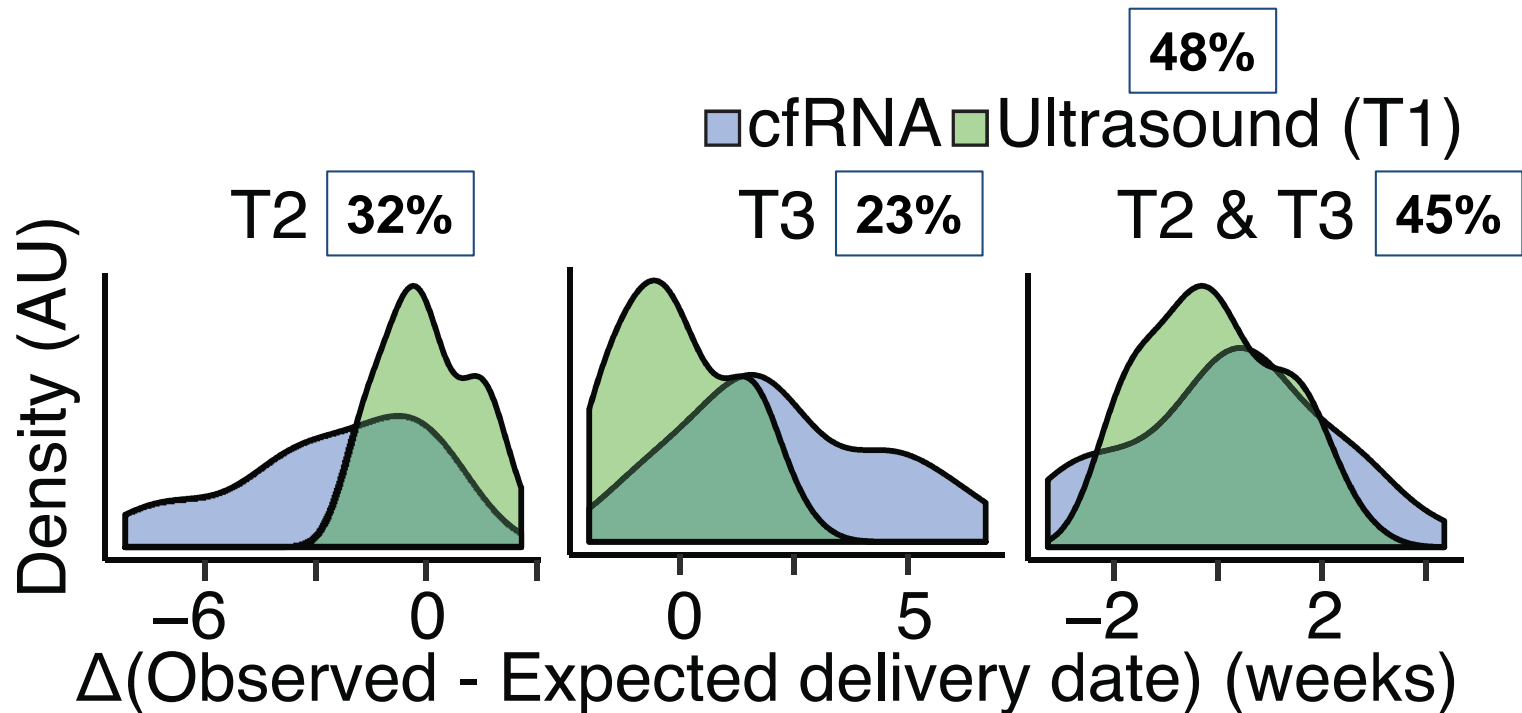
Time to delivery (weeks) = gestational age at collection – gestational age at delivery

The 9 most important genes provide as much predictive power as all 51 genes





# Random forest model predicts gestational age with comparable accuracy to ultrasound



**Developing an accurate and  
inexpensive non-invasive test to  
predict preterm delivery**

# Studying spontaneous preterm delivery

	University of Pennsylvania		University of Alabama at Birmingham	
	Preterm (n=8)	Full-term (n=7)	Preterm (n=5)	Full-term (n=18)
Gest. age (collection)	25.5 ± 2.1	26.8 ± 2.2	24 ± 0	23.9 ± 5.9
Gest. age (delivery)	26.4 ± 2.3	39.2 ± 0.4	30.6 ± 2.4	38.7 ± 1.2
	Early spontaneous labor		Prior spontaneous preterm delivery	

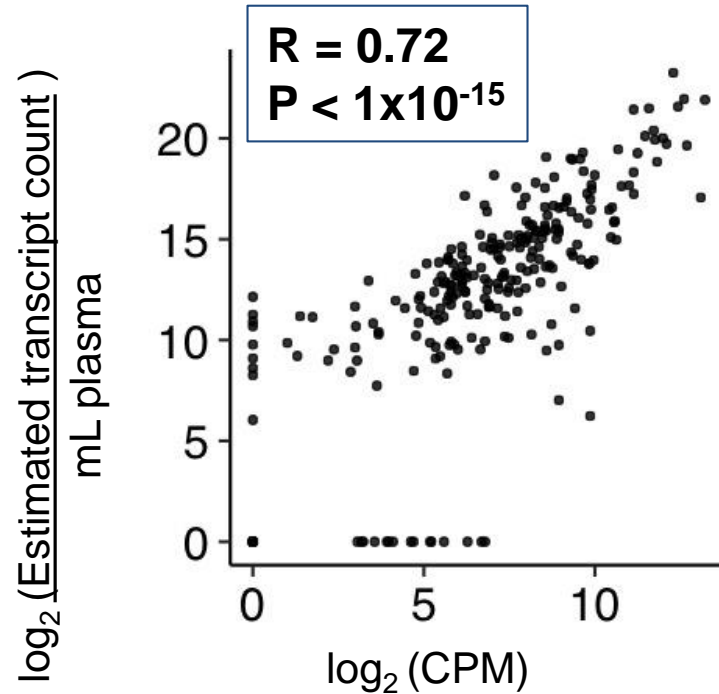
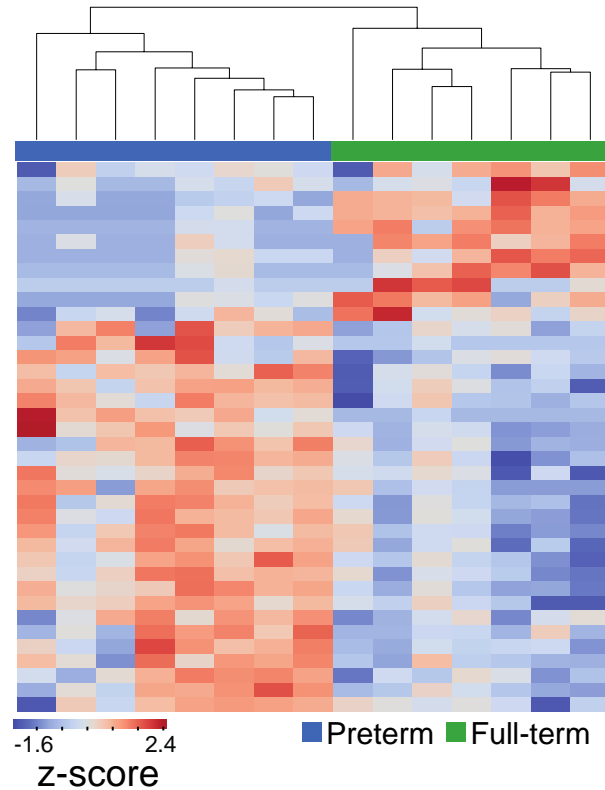
**High-risk  
African-American**

# Random forest model fails to predict spontaneous preterm delivery

	Preterm (n=13)	Full-term (n=25)
RMSE	11.4 weeks	4.3 weeks

**What cfRNA differences if any exist between women who deliver spontaneously preterm and at full-term?**

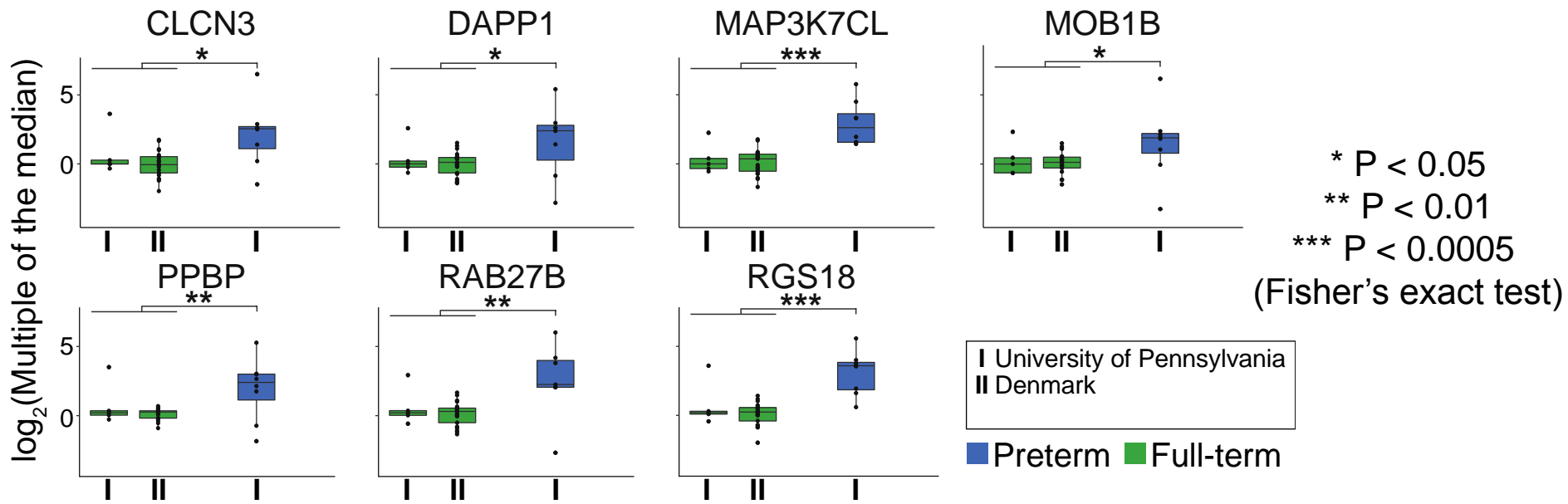
# RNA-seq reveals 38 genes can fully separate spontaneous preterm and full-term pregnancy



$P < 0.001$  (exact test, likelihood ratio test, and quasi-likelihood F-test)

# Developing a risk heuristic for preterm birth

$$\text{Multiple of the median} = \frac{\frac{\text{cfRNA transcript count}}{\text{mL plasma}}}{\text{Full-term median}_{\text{Trimester/Cohort}}}$$



# Developing a risk heuristic for preterm birth

CLCN3, DAPP1, MAP3K7CL, MOB1B, PPBP, RAB27B, RGS18

Gene 1

Gene 2

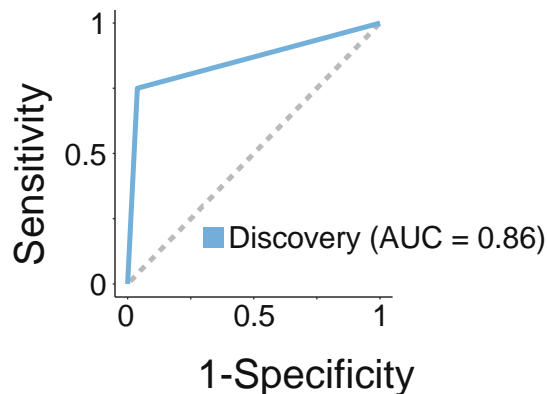
Gene 3

> 2.5 multiple of the median?

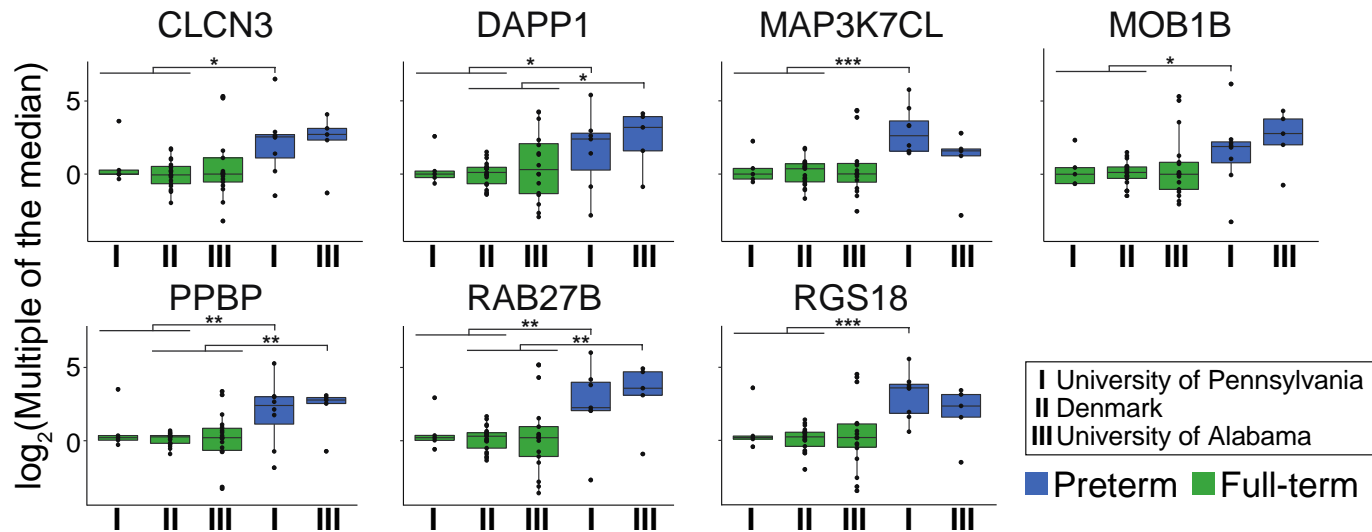
>75% true positive rate?

<5% false positive rate?

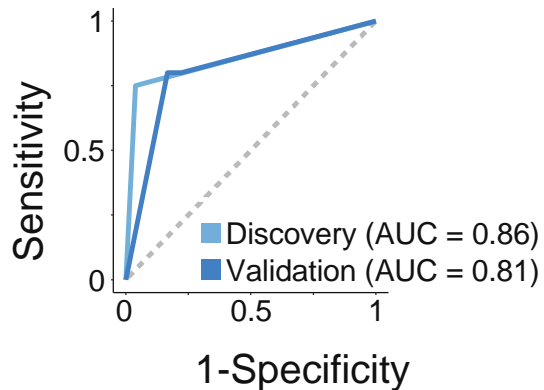
Subject	Combo 1	Combo 2	...	Combo 13	Elevated fraction
1	Yes	No	...	Yes	0.8



# Validating risk heuristic for preterm birth



\*  $P < 0.05$   
\*\*  $P < 0.01$   
\*\*\*  $P < 0.0005$   
(Fisher's exact test)



**Accurate prediction up to 2 months in advance of delivery**



# cfRNA-based risk heuristic has high positive predictive value for high risk women

	AUC	Positive predictive value (%)	Gestational age at testing
cfRNA	0.81	80	< 2 months from delivery
Mass spectrometry <sup>1</sup>	0.67	-	19-21 weeks
Cervical length <sup>2</sup>	-	21	<14 days from delivery
Fetal fibronectin <sup>3</sup>	0.66	46.2	<14 days from delivery

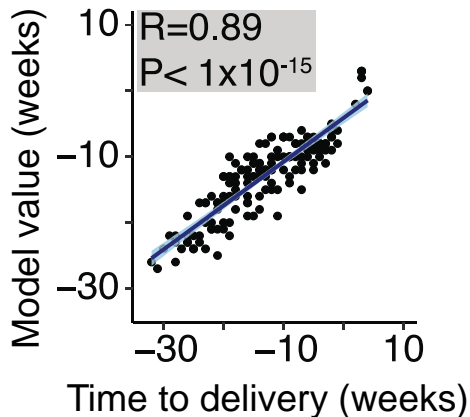
**Questions remain – Does this validate in larger, more ethnically diverse cohorts? What about women who deliver later preterm?**

<sup>1</sup> Saade *et al.* 2016. <sup>2</sup> R. Arisoy *et al.* 2012. <sup>3</sup> Abbott *et al.* 2013.

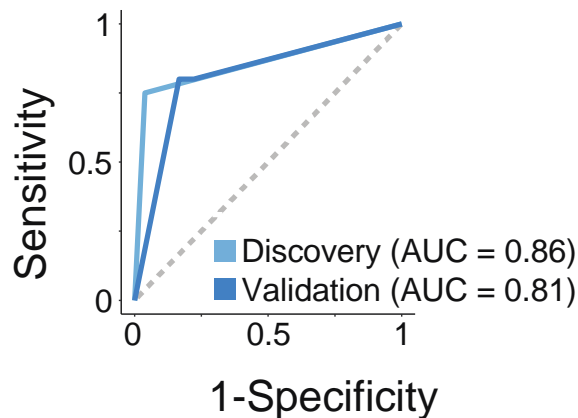
# We can use cfRNA to make accurate and affordable non-invasive diagnostics

## RT-qPCR panel

Gestational age (9 genes)



Risk of spontaneous preterm delivery (7 genes)



# Future work

**Validate previous findings**

**Discover novel cfRNA markers across a spectrum of ethnicities and delivery dates**

**Identify causes of and consequently targeted therapies for spontaneous preterm delivery**

# Thank you!



BILL &  
MELINDA  
GATES  
*foundation*



Stephen Quake  
Thuy Ngo  
Joan Camunas-Soler  
Whitney Combes  
Vickie Lin

Mads Melbye  
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Norma Neff  
Jennifer Okamoto  
Wenying Pan

Michal Elovitz  
Katheryne Downes  
Joseph Biggio

Keli Liu  
Robert Tibshirani

+ the many people at all the  
collection sites whose names I don't  
have handy. Thank you!!