Published: 19 February 2021

Molecular Cancer IF=27

Genome-wide cell-free DNA methylation analyses improve accuracy of non-invasive diagnostic imaging for early-stage breast cancer

Presented by: Reza Khayami

Breast cancer

The most common cancer in women worldwide.

Detection:

- Mammogram
- Ultrasound

routinely administered to detect early BC in asymptomatic females, but prone to underestimation or over-diagnosis

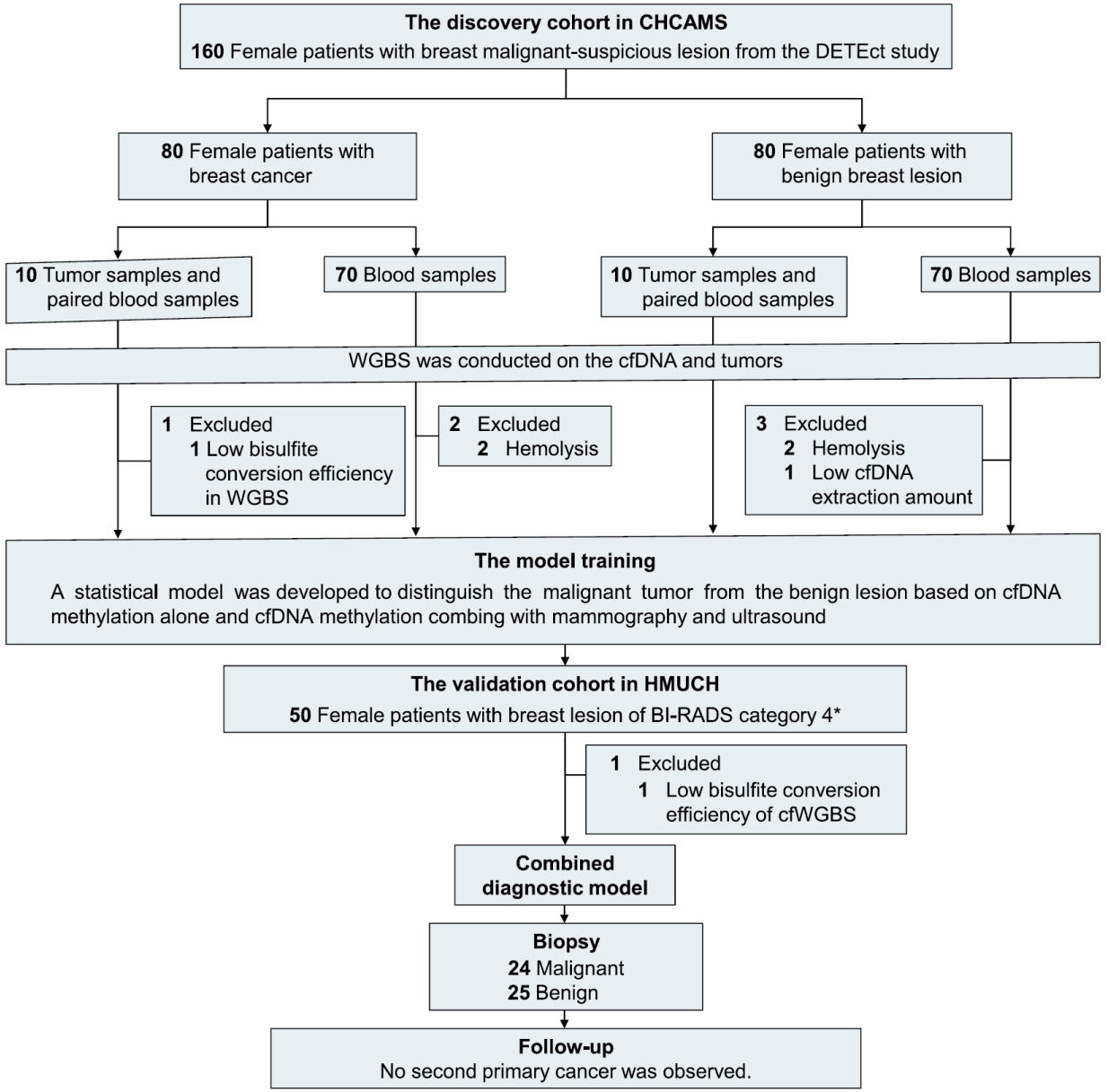
BI- RADS ASSESSMENT CATEGORIES

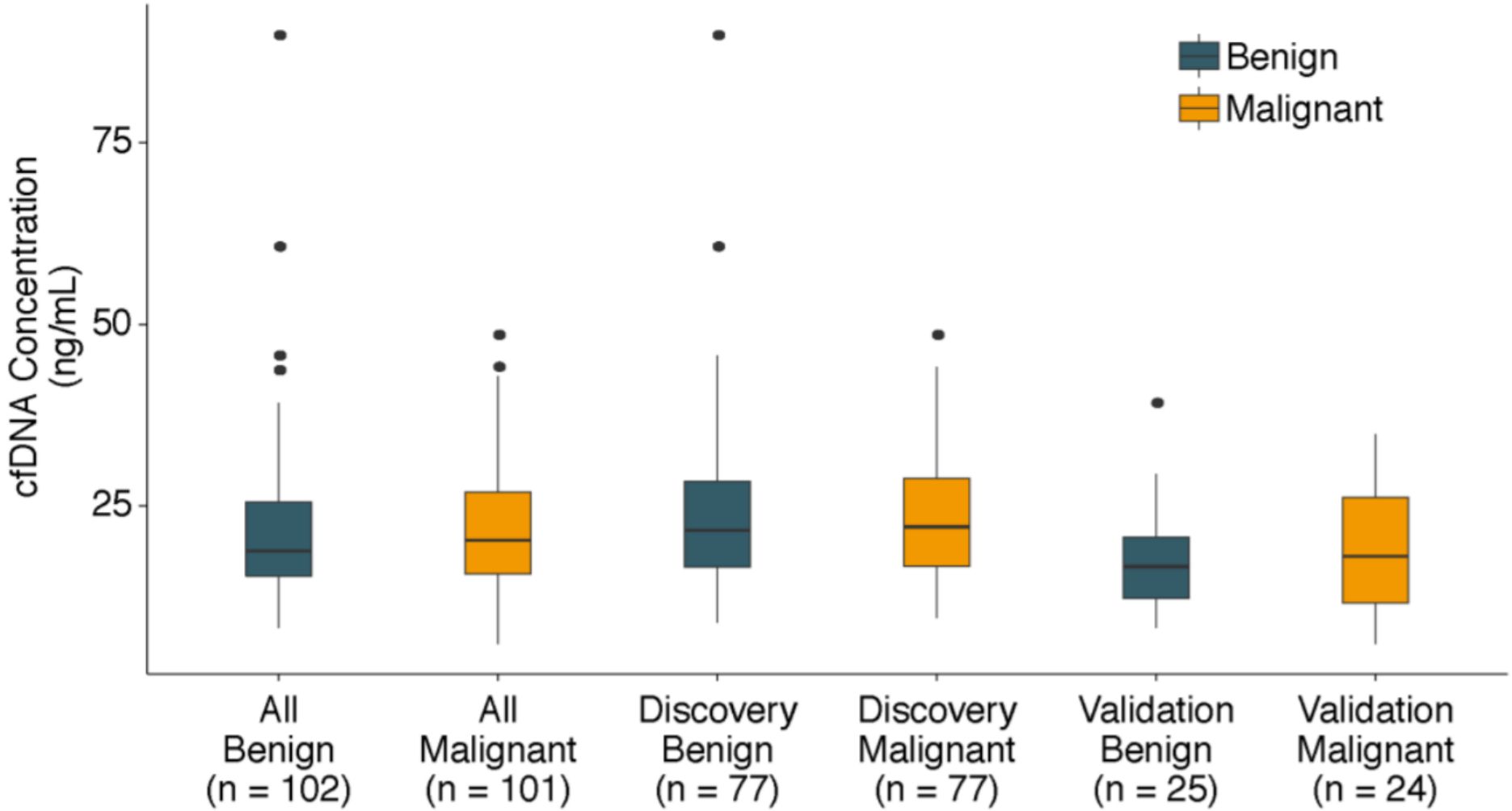
	Category	Recommended Action	Likelihood of Cancer
0	Incomplete	Need additional views / imaging to further evaluate	N/A
1	Negative	Continue routine annual screening	Essentially 0%
2	Probably benign	Continue routine annual screening	Essentially 0%
3	Benign	Short interval follow-up suggested (6 months)	<2% probability of malignancy
4	Suspicious for malignancy	Biopsy should be considered	* 4A: low suspicion for malignancy (2-9%) * 4B: moderate suspicion for malignancy (10-49%) * 4C: high suspicion for malignancy (50-94%)
5	Highly suggestive of malignancy	Biopsy required	Proven malignancy
6	Known biopsy-proven malignancy	Confirmed biopsy and treatment planning	>95% probability of malignancy

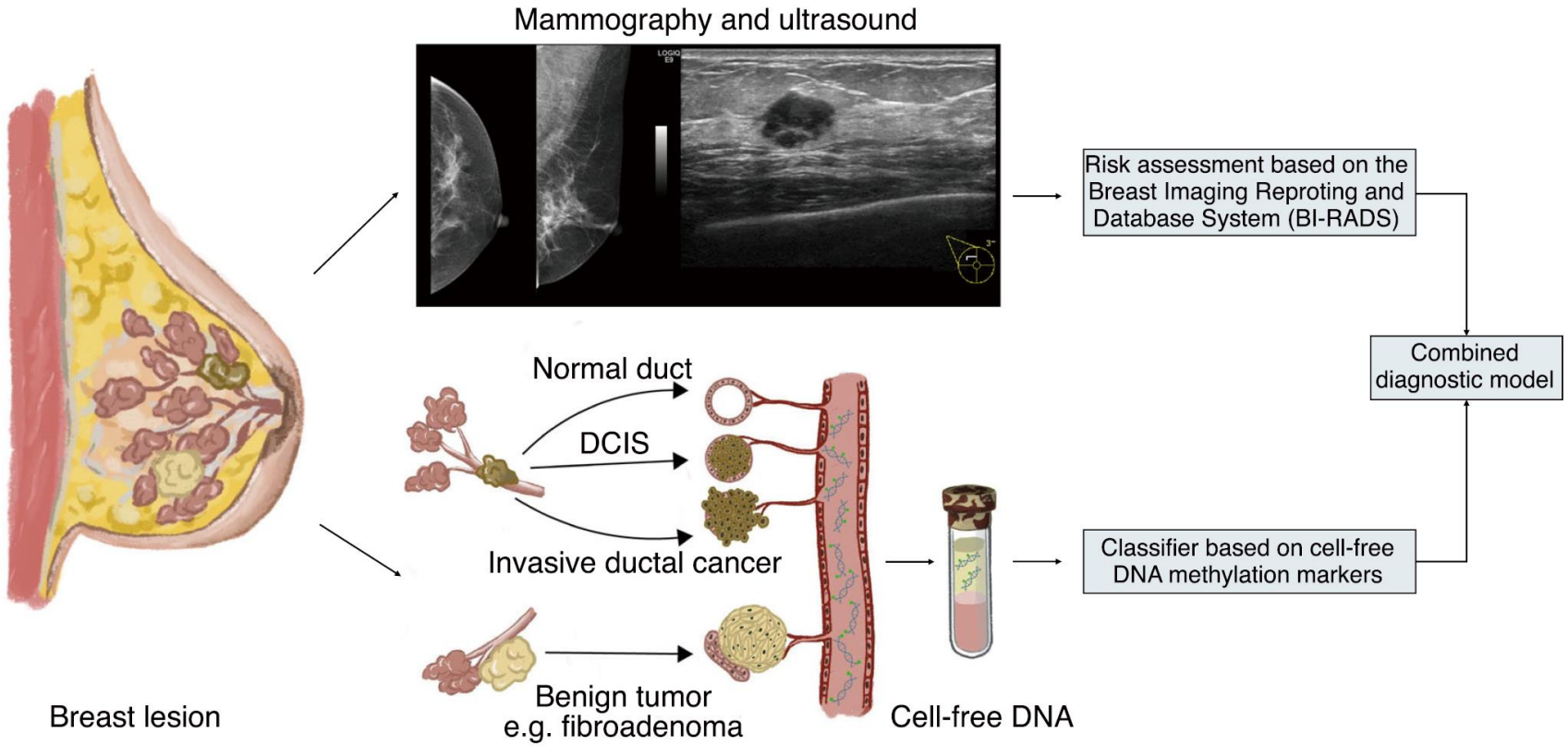
Why methylation?

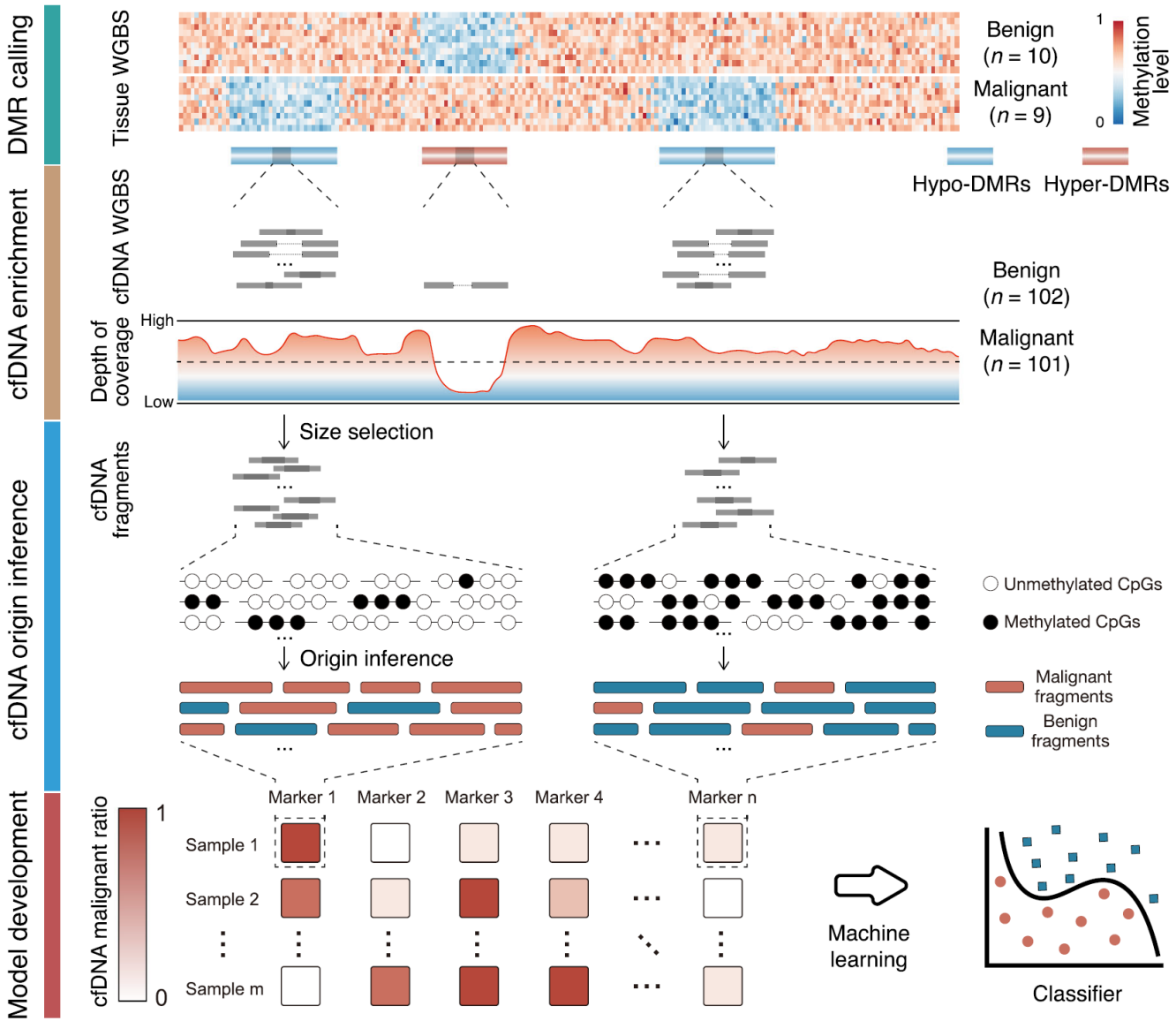
The lack of multiple common mutations in BC has limited the sensitivity of mutation-based ctDNA detection

Combining liquid biopsy with diagnostic imaging

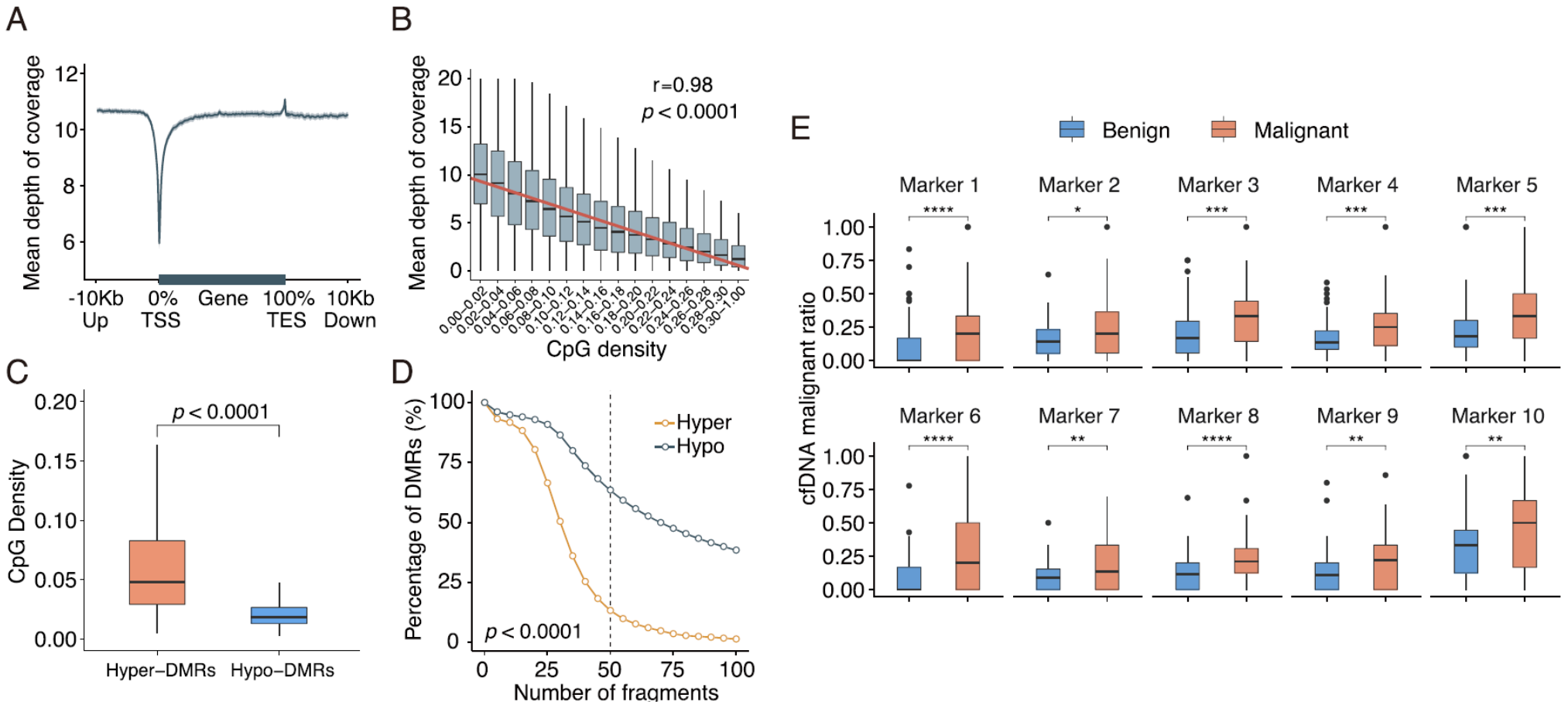

The discovery cohort in CHCAMS
160 Female patients with breast malignant-suspicious lesion from the DETEct study
80 Female patients with breast cancer
80 Female patients with benign breast lesion
10 Tumor samples and paired blood samples
70 Blood samples
10 Tumor samples and paired blood samples
70 Blood samples
WGBS was conducted on the cfDNA and tumors
1 Excluded
1 Low bisulfite conversion efficiency in WGBS
2 Excluded
2 Hemolysis
3 Excluded
2 Hemolysis
1 Low cfDNA extraction amount
The model training
A statistical model was developed to distinguish the malignant tumor from the benign lesion based on cfDNA methylation alone and cfDNA methylation combing with mammography and ultrasound
The validation cohort in HMUCH
50 Female patients with breast lesion of BI-RADS category 4*
1 Excluded
1 Low bisulfite conversion efficiency of cfWGBS
Combined diagnostic model
Biopsy
24 Malignant
25 Benign
Follow-up
No second primary cancer was observed.


Benign
Malignant
cfDNA Concentration (ng/mL)
75
50
25
All Benign (n = 102)
All Malignant (n = 101)
Discovery Benign (n = 77)
Discovery Malignant (n = 77)
Validation Benign (n = 25)
Validation Malignant (n = 24)


Mammography and ultrasound
Risk assessment based on the Breast Imaging Reproting and Database System (BI-RADS)
Combined diagnostic model
Normal duct
DCIS
Invasive ductal cancer
Benign tumor
e.g. fibroadenoma
Classifier based on cell-free DNA methylation markers
Breast lesion
Cell-free DNA

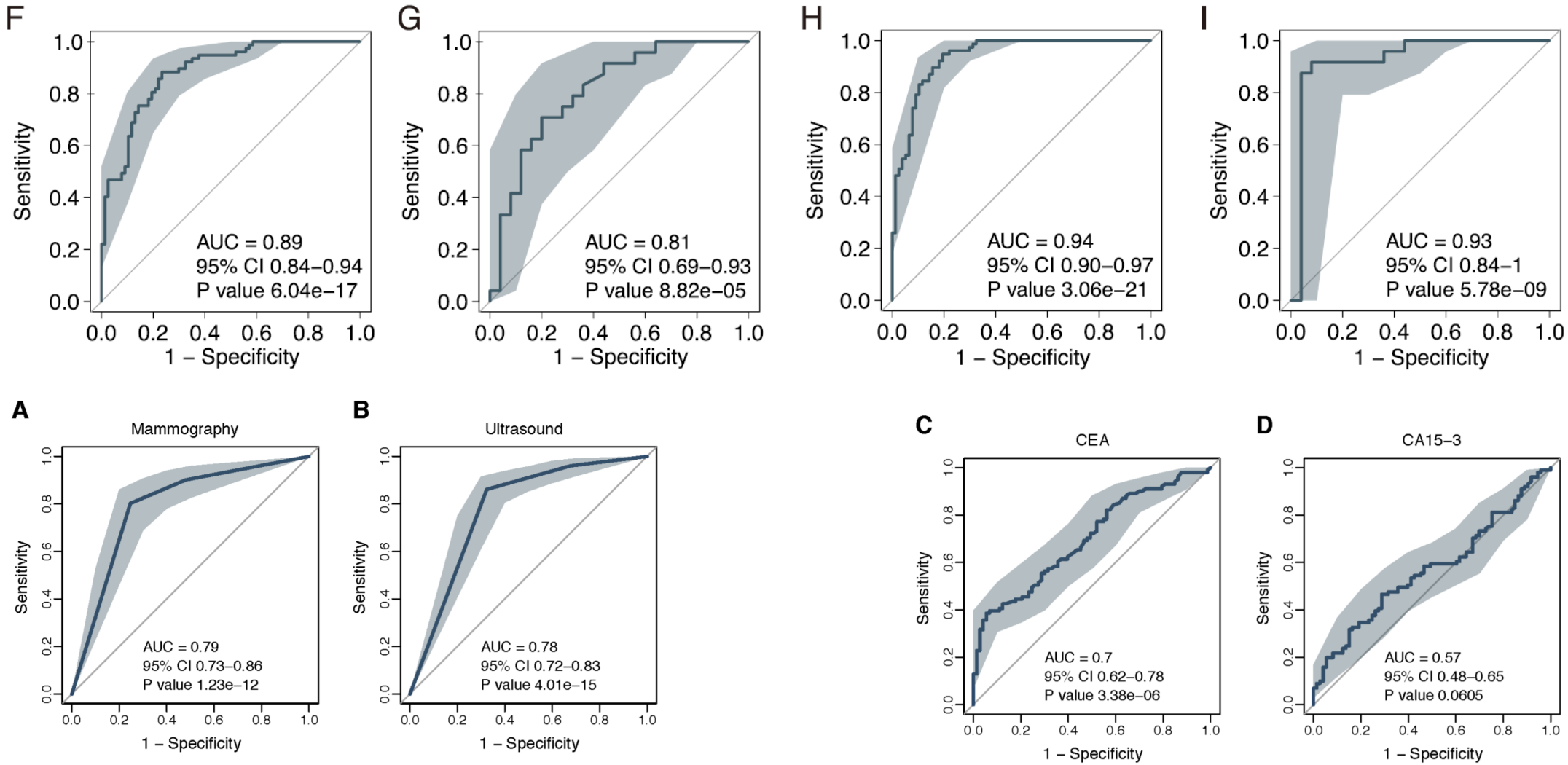


5613 hyper-DMRs
51,962 hypo-DMRs


A
Mean depth of coverage
12
10
8
6
-10Kb Up
0% TSS
Gene
100% TES
10Kb Down
B
Mean depth of coverage
20
15
10
5
0
r=0.98
p < 0.0001
CpG density
C
CpG Density
0.20
0.15
0.10
0.05
0.00
p < 0.0001
Hyper−DMRs
Hypo−DMRs
D
Percentage of DMRs (%)
100
75
50
25
0
Hyper
Hypo
p < 0.0001
0
25
50
75
100
Number of fragments
E
Benign
Malignant
Marker 1
Marker 2
Marker 3
Marker 4
Marker 5
Marker 6
Marker 7
Marker 8
Marker 9
Marker 10
cfDNA malignant ratio
1.00
0.75
0.50
0.25
0.00

Methylation markers

- four functional genes (*RYR2*, *RYR3*, *GABRB3*, and *DCDC2C*)
- two lncRNAs (AC096570.1 and LINC00923)


F
Sensitivity
1 – Specificity
AUC = 0.89
95% CI 0.84–0.94
P value 6.04e–17
G
Sensitivity
1 – Specificity
AUC = 0.81
95% CI 0.69–0.93
P value 8.82e–05
H
Sensitivity
1 – Specificity
AUC = 0.94
95% CI 0.90–0.97
P value 3.06e–21
I
Sensitivity
1 – Specificity
AUC = 0.93
95% CI 0.84–1
P value 5.78e–09
A
Mammography
Sensitivity
1 – Specificity
AUC = 0.79
95% CI 0.73–0.86
P value 1.23e–12
B
Ultrasound
Sensitivity
1 – Specificity
AUC = 0.78
95% CI 0.72–0.83
P value 4.01e–15
C
CEA
Sensitivity
1 – Specificity
AUC = 0.7
95% CI 0.62–0.78
P value 3.38e–06
D
CA15–3
Sensitivity
1 – Specificity
AUC = 0.57
95% CI 0.48–0.65
P value 0.0605

Thank you

"Life is 10% what happens to us and 90% how we react to it."
— Leslie Medley-Russell, two times ovarian cancer survivor