

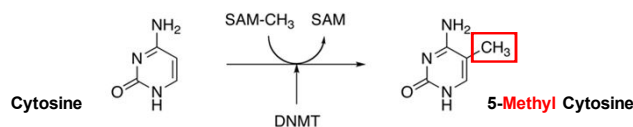
Identification of **age-predictive epigenetic markers** in forensically relevant body fluids

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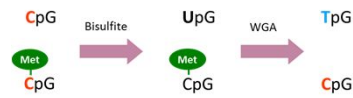
Forensic Age Estimation

- **Age** is an externally visible characteristic that is valuable for **predicting individual's appearance**
- Telomere length, accumulation of mutations and changes in gene expression are correlated with age
- **DNA methylation** is the current most promising age-predictive biomarker

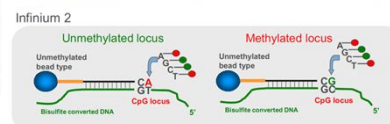
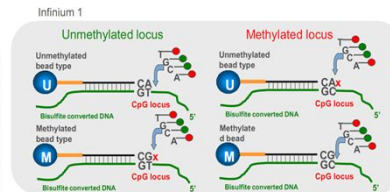


Pipeline of CpG Marker Identification

1. Bisulfite conversion

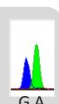


2. Genome-wide DNA methylation profiling



HumanMethylation27 BeadChip Array (Illumina)
HumanMethylation450 BeadChip Array

Candidate marker test:
Pyrosequencing
MassARRAY
Methylation SNaPshot



3. Validation of selected markers

Identification of Age-Related CpGs

- DNA methylation was analyzed in 12 each for blood, saliva and semen samples with an age of 20-59 years
- Linear regression was performed to test for age-association of DNA methylation at each CpG unit

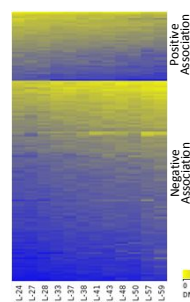


Table. Significant probes from HumanMethylation450 BeadChip

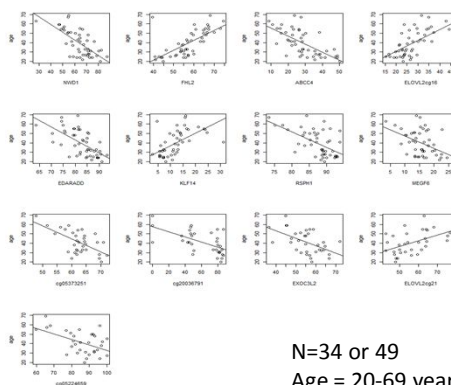
	Blood	Saliva	Semen
Quality-filtered probes	474,546	476,002	476,585
p < 0.01	9,429	30,060	10,892
p < 0.01 & diff (Max, Min) > 0.1	1,445	23,278	4,382
p < 0.01 & r-squared > 0.7 & diff > 0.1	159	628	746

← DNA methylation of 159 CpGs in 12 blood samples

Validation of Correlated CpGs in Blood

- Age association of 26 CpGs with a high R^2 value and a large max-min difference was tested using methylation SNaPshot

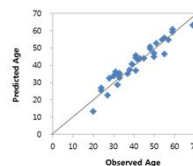
	Estimate(SE)	P-value
NWD1	-0.8315(0.1291)	<.0001
FHL2	1.0542(0.1691)	<.0001
ABCC4	-0.8525(0.1405)	<.0001
ELOVL2cg16	1.1674(0.1982)	<.0001
EDARADD	-1.3901(0.2440)	<.0001
KLF14	1.3295(0.2756)	<.0001
RSPH1	-1.6555(0.3499)	<.0001
MEGF6	-1.3197(0.3508)	0.0005
cg05373251	-1.4032(0.3178)	0.0001
cg20036791	-0.2724(0.0669)	0.0003
EXOC3L2	-0.7817(0.2263)	0.0016
ELOVL2cg21	0.6117(0.1817)	0.0020
cg05224659	-0.5913(0.1837)	0.0029



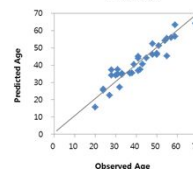
Age-Predictive Models in Blood

$$\text{Age} = \beta_0 + \beta_1 \times \text{CpG}_1 + \beta_2 \times \text{CpG}_2 + \dots$$

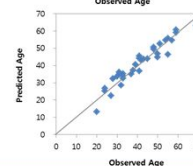
	Coefficients		
	Model-1	Model-2	Model-3
(Intercept)	63.942	82.161	117.842
NWD1	0.011		-0.197
FHL2	0.801	0.941	0.448
ABCC4	-0.168		
ELOVL2cg16	0.128		0.430
EDARADD	0.052		-0.510
KLF14	0.141		0.320
RSPH1	-0.539	-0.614	-0.743
MEGF6	-0.266	-0.404	
cg05373251	-0.652	-0.517	
cg20036791	-0.086	-0.075	
EXOC3L2	0.101		
ELOVL2cg21	0.079		
cg05224659	0.126		



Model-1
Spearman's Rho = 0.965
N = 34
Average absolute difference
between observed and
predicted age
= 2.8



Model-2
Spearman's Rho = 0.947
N = 34
Average absolute difference
= 3.4

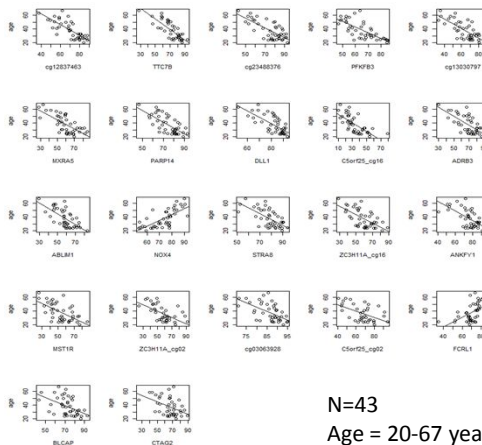


Model-3
Spearman's Rho = 0.916
N = 49
Average absolute difference
= 4.2

Validation of Correlated CpGs in Semen

	Estimate(SE)	P-value
cg12837463	-0.7909(0.0987)	<.0001
TTC7B	-0.7772(0.0920)	<.0001
cg23488376	-0.7107(0.1031)	<.0001
PFKFB3	-0.8160(0.1221)	<.0001
cg13030797	-0.6863(0.1028)	<.0001
MXRA5	-0.6665(0.1046)	<.0001
PARP14	-0.8492(0.1349)	<.0001
DLL1	-0.9218(0.1505)	<.0001
C5orf25	-0.6381(0.1042)	<.0001
ADRB3	-0.6600(0.1107)	<.0001
ABLIM1	-0.8020(0.1387)	<.0001
NOX4	0.8611(0.1355)	<.0001
STRA8	-0.8313(0.1531)	<.0001
ZC3H11A	-0.6128(0.1158)	<.0001
ANKFY1	-0.6945(0.1346)	<.0001
MST1R	-0.5524(0.1108)	<.0001
ZC3H11A-cg02	-0.4537(0.1040)	0.0010
cg03063928	-1.2392(0.2794)	<.0001
C5orf25-cg02	-0.4561(0.1212)	<.0001
FCRL1	0.7091(0.1859)	<.0001
BLCAP	-0.5821(0.1648)	0.0026
CTAG2	-0.5821(0.1648)	0.0010

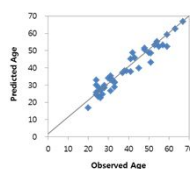
□ 27 CpGs were tested in semen



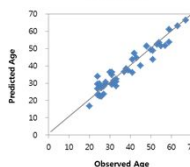
N=43
Age = 20-67 years

Age-Predictive Models in Semen

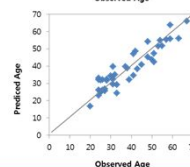
	Coefficients		
	Model-1	Model-2	Model-3
(Intercept)	48.659	27.289	39.844
cg12837463	-0.195		
TTC7B	-0.171		
cg23488376	-0.408	-0.614	-0.516
PFKFB3	-0.068		-0.155
cg13030797	-0.010		
MXRA5	-0.024		
PARP14	-0.197	-0.377	
DLL1	0.094		
C5orf25	-0.228	-0.248	
ADRB3	0.394	0.351	
ABLIM1	-0.015		
NOX4	0.577	0.766	0.642
STRA8	0.198		
ZC3H11A	-0.091	0.219	
ANKFY1	0.057		
MST1R	0.031		
ZC3H11A-cg02	0.236		
cg03063928	-0.471		
C5orf25-cg02	-0.077		
FCRL1	0.144		
BLCAP	0.226		
CTAG2	-0.173		



Model-1
Spearman's Rho = 0.930
N = 40
Average absolute difference between observed and predicted age = 2.7



Model-2
Spearman's Rho = 0.929
N = 43
Average absolute difference between observed and predicted age = 2.9

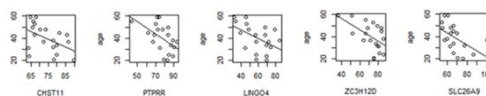


Model-3
Spearman's Rho = 0.880
N = 43
Average absolute difference between observed and predicted age = 4.0

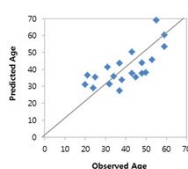
Validation of Correlated CpGs in Saliva

- 23 CpGs were tested in saliva, but only a few showed age association probably due to the use of fragmented DNA for BeadChip analysis as well as for methylation SNaPshot

	Estimate(SE)	P-value
CHST11	-0.6964(0.3097)	0.0373
PTPRR	-0.5497(0.2073)	0.0162
LINGO4	-0.3832(0.1673)	0.0343
ZC3H12D	-0.5116(0.1819)	0.0115
SLC26A9	-0.5372(0.2240)	0.0282



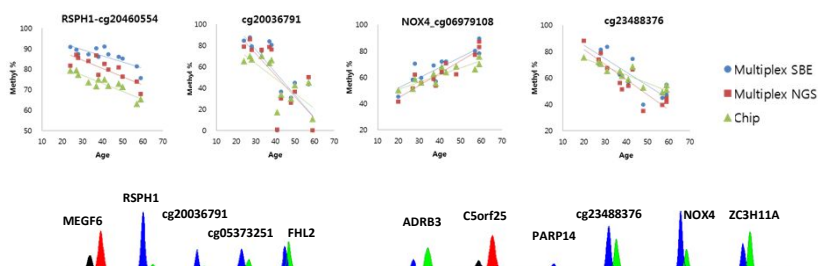
	Coefficients
(Intercept)	111.651
CHST11	-0.223
PTPRR	0.014
LINGO4	-0.093
ZC3H12D	-0.382
SLC26A9	-0.351



Model-1
Spearman's Rho = 0.742
N = 20
Average absolute difference between observed and predicted age = 7.6

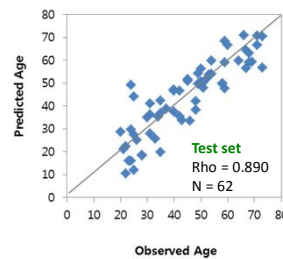
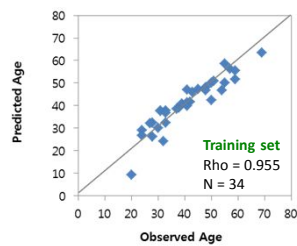
A Multiplex Example for Age Estimation

- A **multiplex** that enables the **convenient and reliable quantitative analysis of methylation** at selected CpG sites will facilitate the application of DNA methylation to forensic age estimation



A Multiplex Example for Age Estimation

- A multivariate linear regression model was adjusted to facilitate age prediction based on 4 CpGs using multiplex methylation SNaPshot. The average absolute difference between the predictive and observed age was 3.5 years in a training set (N = 34) and 6.4 years in a test set (N = 62)



Summary

- We selected five to six CpG sites and built a regression model for age prediction in each body fluid. Each model facilitates age predictions with an average absolute difference between the predictive and observed age of less than 8.
- Development of a multiplex system that enables a less costly, faster, convenient and reliable quantitative assay of DNA methylation at a few selected CpG sites will facilitate the application of DNA methylation to forensic age estimation.

Thank you
for your attention

