

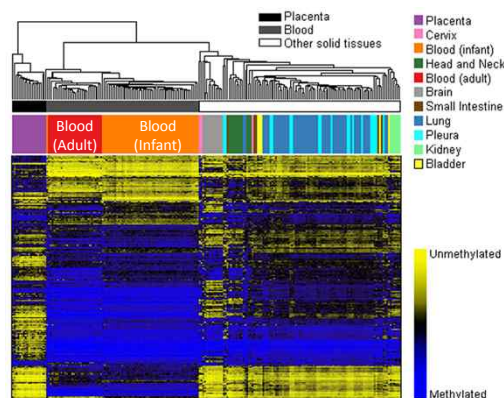
DNA methylation and age prediction in semen

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DNA methylation across tissues

- Many age-related DNA methylation changes depend on tissue type



Unsupervised clustering of average beta values in normal human tissues.
Christensen et al. PLoS Genet (2009)

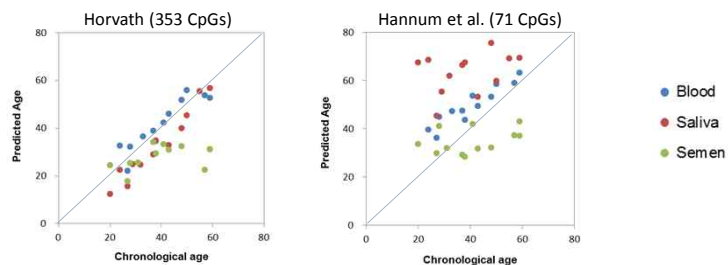
DNA methylation-base age prediction

- Age-predictive models based on the use of blood or even across a broad spectrum of tissues have been reported

	Age signatures	Tissue	Error (years)
Bocklandt et al. (2011)	EDARADD, TOM1L1, NPTX2	Saliva	5.2
Garagnani et al. (2012)	ELOVL2, FHL2, PENK	Blood	-
Weidner et al. (2014)	ITGA2B, ASPA, PDE4C	Blood	4.3
Zbiec-Piekarska et al. (2015)	ELOVL2, C1orf132, TRIM59, KLF14, FHL2	Blood	3.9
Huang et al. (2015)	ASPA, ITGA2B, NPTX2	Blood	7.9
Hannum et al. (2013)	71 CpGs from Illumina's beadchip array	Blood	3.9
Horvath (2013)	353 CpGs from Illumina's beadchip array	Somatic tissues	3.6

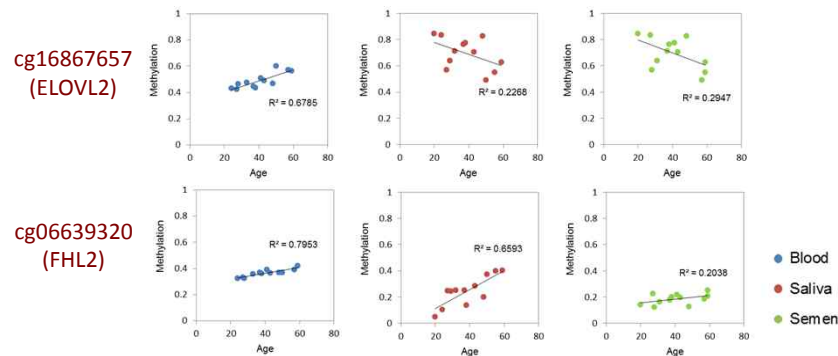
Age prediction in different body fluids

- Age predictive values for 36 body fluid samples (GSE59505) were compared between the two age-predictive models suggested by Horvath and Hannum et al.
- The two models using many CpGs from the Illumina's BeadChip array showed considerable accuracy in blood



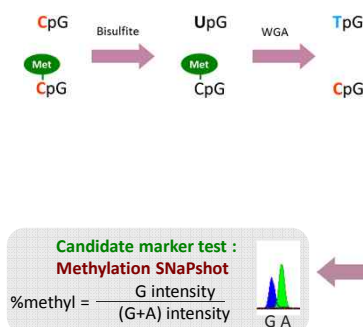
DNA methylation in different body fluids

- Strong age correlation of DNA methylation at **cg16867657 (ELOVL2)** and **cg06639320 (FHL2)** was observed in the 450K BeadChip array data **from blood but not from semen**



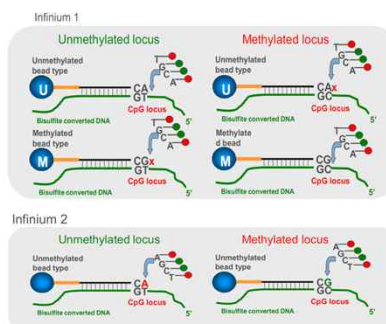
Pipeline of CpG marker identification

1. Bisulfite conversion



3. Validation of selected markers

2. Genome-wide DNA methylation profiling



HumanMethylation450 BeadChip Array (Illumina)

Identification of age-related CpGs

- DNA methylation at 485,000 CpG loci was analyzed in semen samples obtained from 12 individuals aged 20-59
- Univariate linear regression was performed for each CpG to test the association between DNA methylation and age

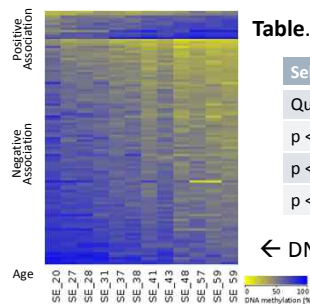


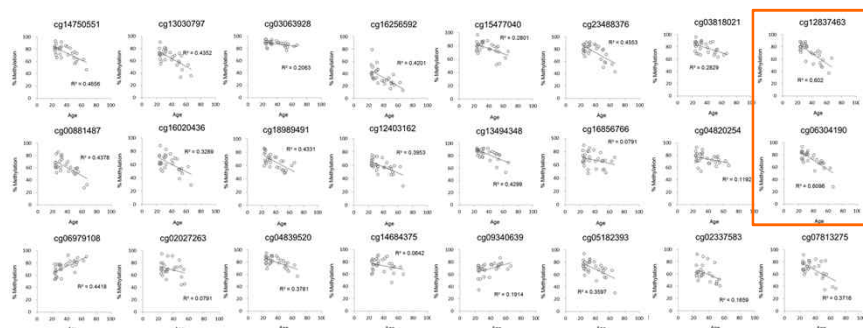
Table. Significant probes from Methylation450 BeadChip

Selection criteria	No. probes
Quality-filtered probes	479,686
$p < 0.01$	10,710
$p < 0.01$ & $r\text{-squared} > 0.7$	1,316
$p < 0.01$ & $r\text{-squared} > 0.7$ & $\text{abs. estimate} > 0.005$	106

← DNA methylation of 106 CpGs in 12 semen samples

Validation of candidate CpGs in semen

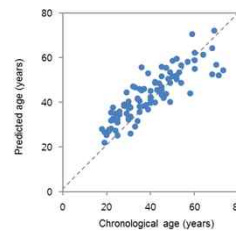
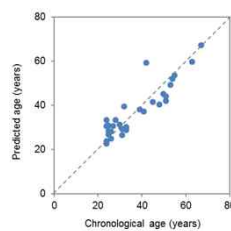
- DNA methylation at 24 CpG marker candidates were obtained from independent 31 semen samples by targeted bisulfite sequencing using methylation SNaPshot



Age-predictive model in semen

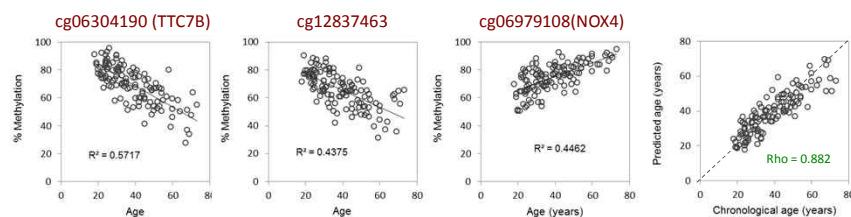
- Stepwise regression, the most popular form of variable selection, produced a model composed of 3 CpGs

Target ID	R-squared	Estimate (n = 31)	P-value	R-squared	RMSE	MAD	Gene symbol
(Intercept)		74.153	0				
cg06304190	0.6096	-0.46	0	0.814	5.835	4.2	TTC7B
cg12837463	0.6020	-0.353	0.002				
cg06979108	0.4418	0.304	0.017				NOX4



Retrained age-predictive model in semen

- Age correlation of the 3 CpGs and predicted versus chronological ages of 125 semen samples



Target ID	Estimate (n = 125)	P-value	R-squared	RMSE	MAD	Gene symbol
(Intercept)	46.240	0				
cg06304190	-0.519	0	0.766	6.690	5.2	TTC7B
cg12837463	-0.178	0.007				
cg06979108	0.541	0				NOX4

Summary

- ❑ The current study is the first report of an age-predictive model for semen
 - ❑ Previously reported age predictors showed considerable prediction accuracy in blood but not in semen
 - ❑ The 3 CpG sites including those in the TTC7B gene and the NOX4 gene were suggested as epigenetic age signatures to be useful for accurate age prediction in semen
 - ❑ Our model which uses only a small number of CpG sites and does not require complex bioinformatics could be more appealing
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Thank you for your attention!
Hope to see you again at
ISFG2017 meeting in Seoul

