

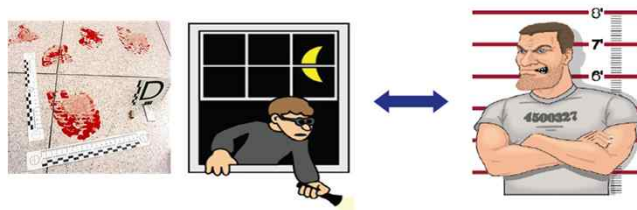
Epigenetic age signatures in the forensically relevant body fluid of semen

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Current Forensic DNA Typing

- Forensic cases -- **matching suspect with evidence**

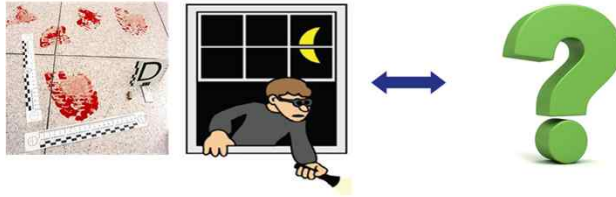


*Involves generation of DNA profiles usually with the same genetic markers (STRs) and then **MATCHING TO REFERENCE SAMPLE***

Picture from www.cstl.nist.gov/strbase/NISTpub.htm

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DNA Mass Screening



I WANT YOU
TO SUBMIT TO A DNA TEST

- The largest known DNA sweep in Germany took place in 1998. More than 15,000 people were tested before the killer of an 11-year-old girl was found.

**Would this be
an invasion of privacy?**

Picture from www.councilforresponsiblegenetics.org/

Forensic Phenotyping

- Forensic phenotyping is expected to be criminally useful in helping **to reduce the number of potential suspects**.



Picture from snapshot.parabon-nanolabs.com

Forensic Phenotyping

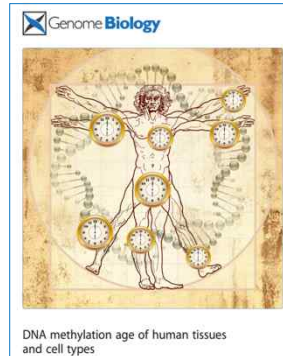
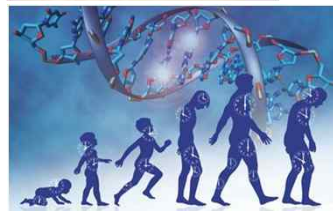
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Picture from snapshot.parabon-nanolabs.com

Age Prediction

- **Age as EVC (externally visible characteristics)** is expected to provide investigative lead to track unknown suspect or to identify missing persons **regardless of ethnicity**



Horvath. Genome Biol (2013)

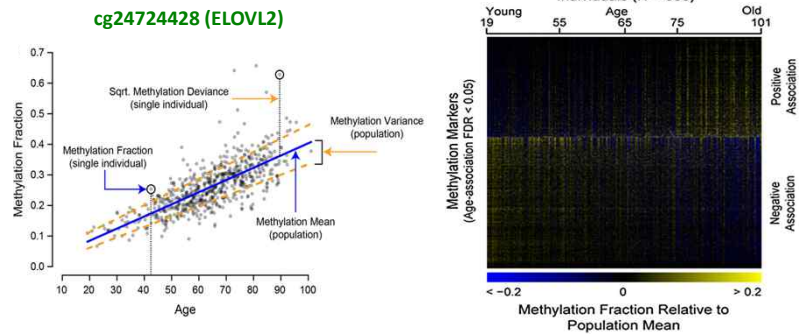
DNA methylation-based age prediction

- 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 HumanMethylation450 array	Blood	3.9
Horvath (2013)	353 CpGs from HumanMethylation27 array	Somatic tissues	3.6

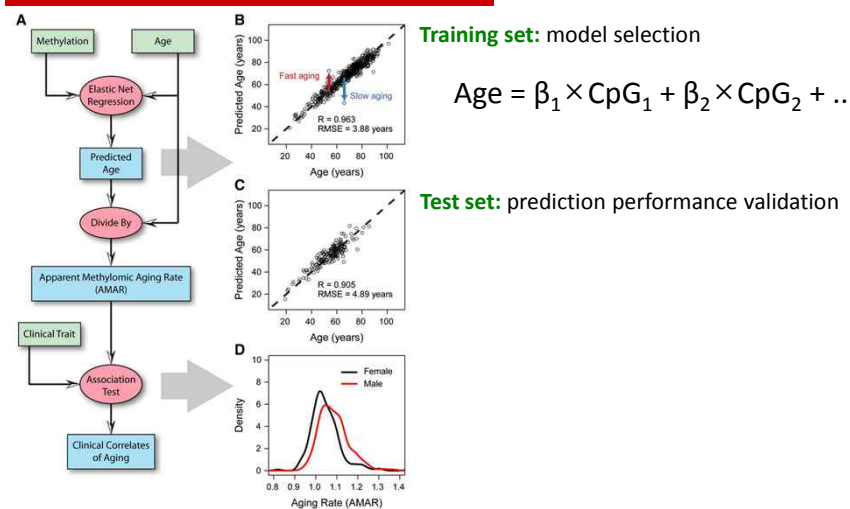
Age-related DNA Methylation Changes

- There are markers which have significant association between methylation fraction and age



Hannum et al. Mol Cell (2013)

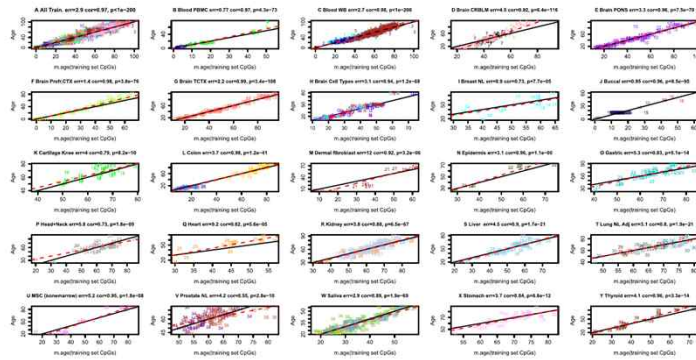
Age Predictive Model Construction



Hannum et al. Mol Cell (2013)

Age Predictive Model by Horvath

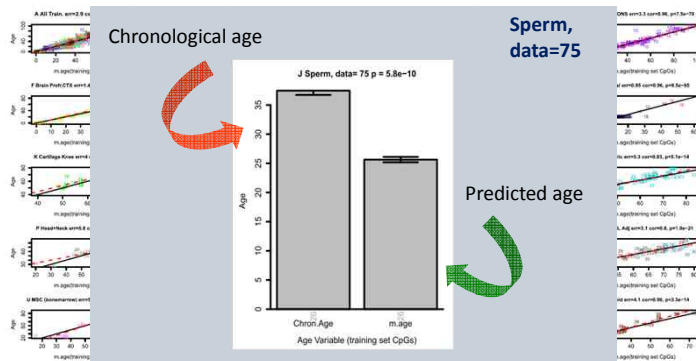
- Age predictor suggested by Horvath could be applied across a broad spectrum of tissues but not to sperm cells



Horvath, Genome Biol (2013)

Age Predictive Model by Horvath

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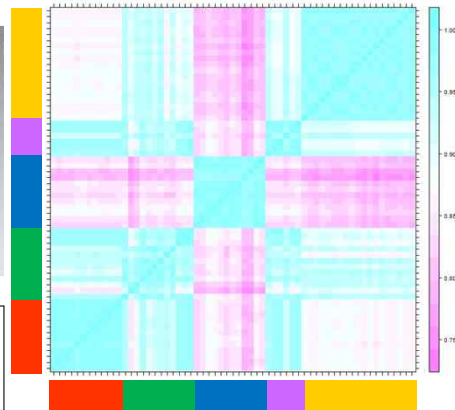
Horvath, Genome Biol (2013)

Age prediction in Semen

- DNA profile can be obtained from semen of unknown suspect



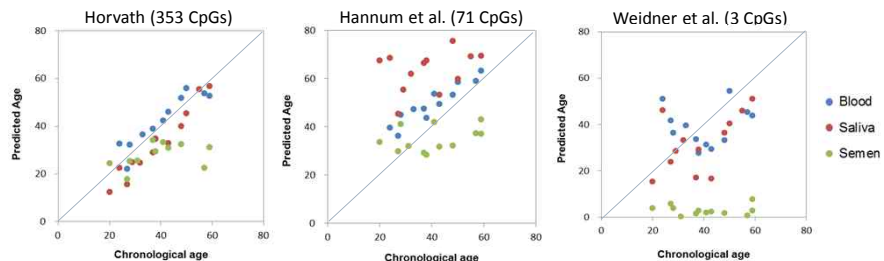
DNA methylation profiles
(GSE59505, GSE51954)



Picture from <http://cartoonistsatish.blogspot.kr/2010/12/wikileaks-founder-julian-assange.html>

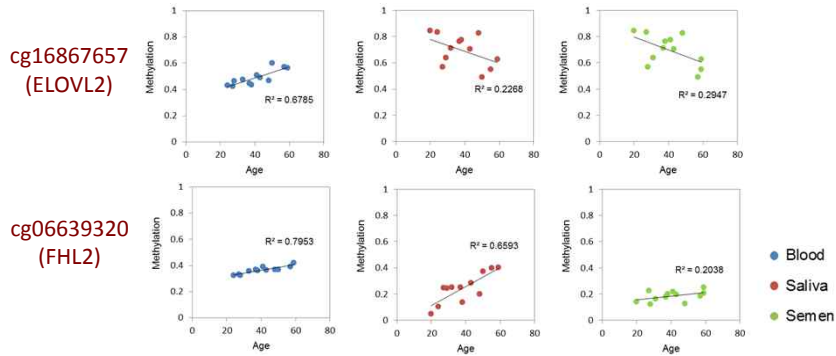
Age prediction in different body fluids

- Age predictive values for 36 body fluid samples (GSE59505) were compared between the three age-predictive models suggested by Horvath (2013), Hannum et al. (2013) and Weidner et al. (2014).



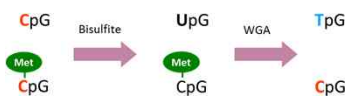
DNA methylation in different body fluids

- 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

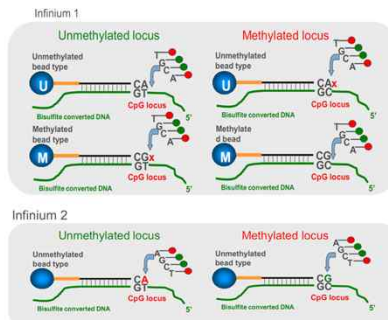


Pipeline of CpG marker identification

1. Bisulfite conversion



2. Genome-wide DNA methylation profiling



HumanMethylation450 BeadChip Array (Illumina)

Candidate marker test : Methylation SNaPshot

$$\% \text{methyl} = \frac{\text{G intensity}}{(\text{G+A}) \text{ intensity}}$$

3. Validation of selected markers

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

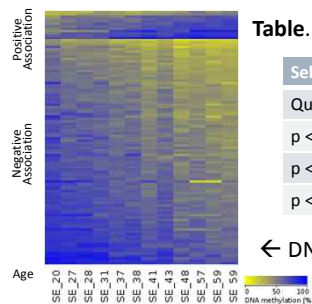


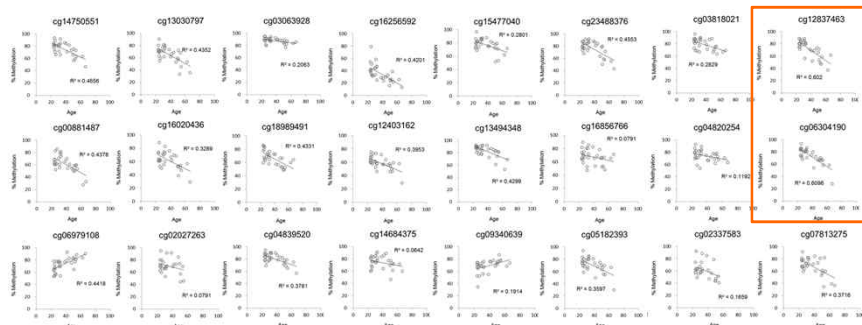
Table. Significant probes from Methylation450 BeadChip

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

← DNA methylation of 106 CpGs in 12 semen samples

Validation of candidate CpGs in semen

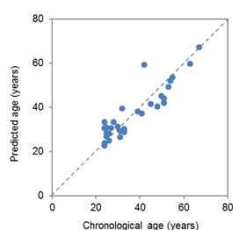
- DNA methylation at **24 CpG marker candidates** were obtained from **independent 31 semen samples** by targeted bisulfite sequencing using **methylation SNaPshot**



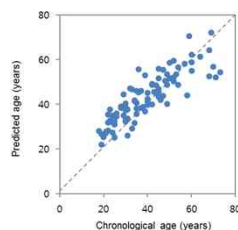
Age-predictive model in semen

- 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				



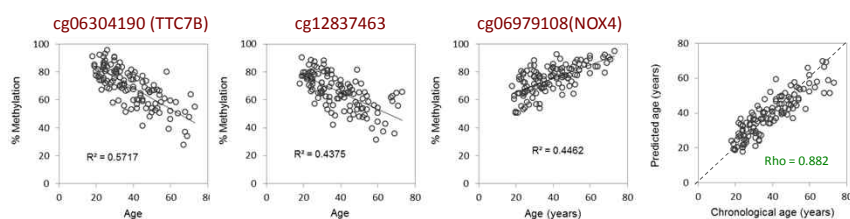
Training set
Rho = 0.832
N = 31
MAD = 4.2



Test set
Rho = 0.882
N = 94
MAD = 6.5

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				

TTC7B and NOX4 Genes

- **NOX4 (NADPH oxidase 4)** is a member of the NOX family of NADPH oxidases, and has been known to protect the vasculature against inflammatory stress.
- **TTC7B (tetra-trycotopeptide repeat domain 7B)** was suggested as a novel risk factor for ischemic stroke, and the region around this gene has been reported to show age-related DNA methylation alteration in the sperm methylome of 2 samples collected from individuals at certain time intervals.

Candidates for Semen Age Prediction

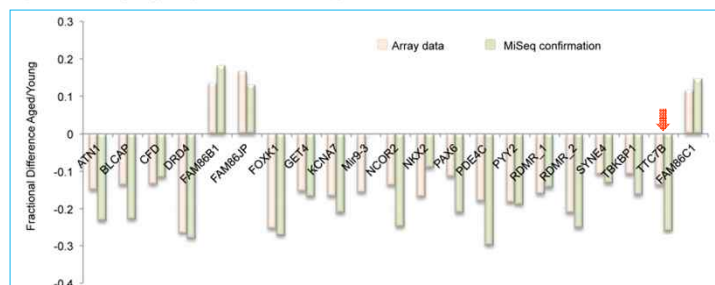
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PLOS GENETICS

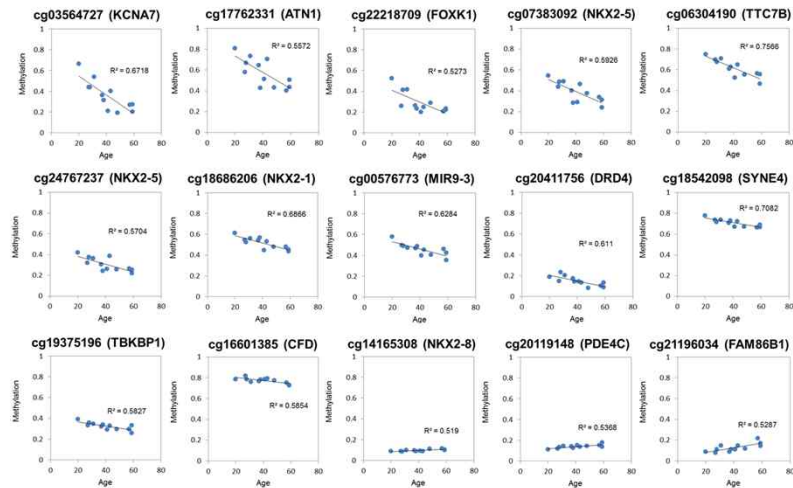
Age-Associated Sperm DNA Methylation Alterations: Possible Implications in Offspring Disease Susceptibility

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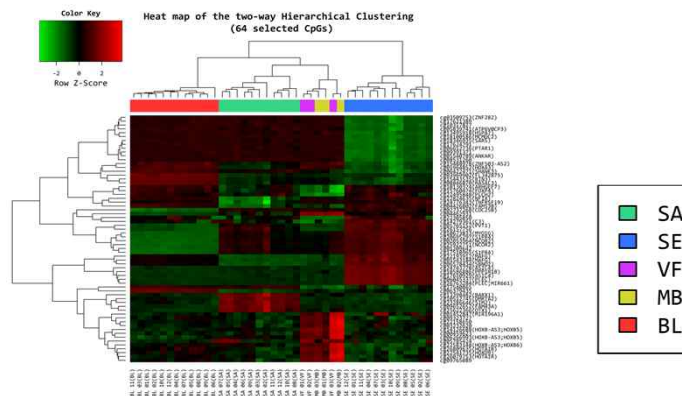


Candidates for Semen Age Prediction



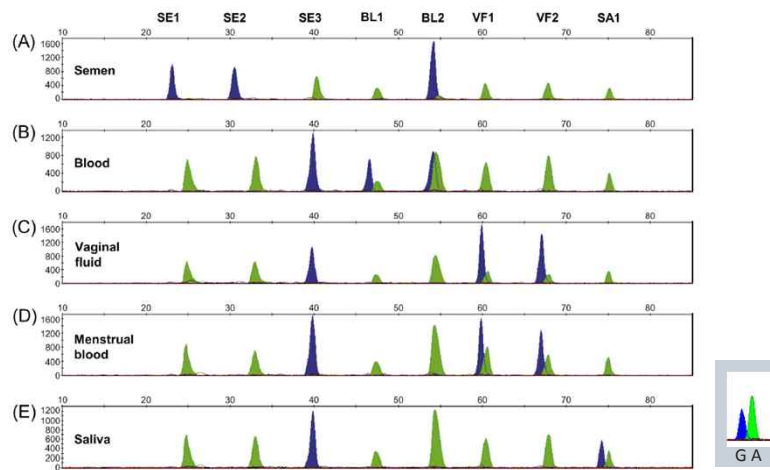
DNA methylation across body fluids

- Tissue-specific DNA methylation changes can be used to differentiate among body fluids



Lee et al. Forensic Sci Int Genet (2015)

DNA Methylation-Based Body Fluid ID



Summary

- ❑ 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 to the investigators
- ❑ DNA methylation analysis will provide additional layer of information to forensic genetics

Acknowledgement

- ❑ Yonsei DNA Profiling Group (<http://forensic.yonsei.ac.kr>)
- ❑ Special thanks to Sang-Eun Jung, Yu-Na Oh, Ajin Choi, Ja Hyun An, Woo Ick Yang and Kyoung-Jin Shin
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