Genome-Wide Methylation Profiling to Identify Body Fluid- and Age-Associated DNA Methylation Changes

Ja Hyun An · Ajin Choi · Sang-Eun Jung · Kyoung-Jin Shin · Woo Ick Yang · Hwan Young Lee

Department of Forensic Medicine, Yonsei University College of Medicine, 50 Yonsei-Ro, Seodaemun-Gu, Seoul 120-752, Korea
Tel: +82-2-2228-2482, e-mail: hylee192@yuhs.ac

Introduction

The ability to predict tissue type and donor's age from molecular profiles of crime scene samples has practical implications in forensics. Recently, DNA methylation profiling has been in the spotlight as a promising new tool for distinguishing between different types of body fluids because of the high specificity, compatibility with existing STR typing protocols, and fit with current forensic casework application. DNA methylation occurs at the 5′-position of the pyrimidine ring of cytosine in CpG dinucleotides and influences DNA stability and transcriptional silencing without changing sequence information. Different cell types have different methylation patterns, and chromosome segments called tDMRs (tissue-specific differentially methylated regions) are known to show different DNA methylation profiles according to cell or tissue type. In addition, growing evidence suggests that DNA methylation is important in cellular senescence and aging. Therefore, research to reveal the association between age and DNA methylation changes is being carried out as well as studies to identify tissue-specific DNA methylation. Here, we analyzed DNA methylation patterns of body fluids (blood, saliva, semen, menstrual blood, and vaginal fluid) from 3-12 individuals aged 20 to 59 each using the Illumina Infinium Human Methylation450 BeadChip array in order to identify body fluid- and age-associated DNA methylation changes, and then carried out gene-specific analysis for selected markers using methylation SNaPshot.

Materials and Methods

Samples

Body fluid samples (venous blood, saliva, semen, menstrual blood and vaginal fluid) were collected from 65 volunteers aged 20 to 69 (49 males and 16 females) using procedures approved by the Institutional Review Board of Severance Hospital, Yonsei University in Seoul, Korea. DNA was extracted from an aliquot of blood, saliva and semen or a single cotton swab of menstrual blood and vaginal fluid using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Extracted DNA was quantified using a Quantifiler® Duo DNA Quantification Kit (Applied Biosystems, Foster City, CA, USA).

HumanMethylation450 BeadChip

We analyzed DNA methylation profile of 485,000 CpG loci in various body fluids DNA from a group of volunteers with different ages, using HumanMethylation450 BeadChip array (Illumina, San Diego, CA, USA). We assayed 42 samples, including each 12 samples of blood, saliva, semen (age range 20-59) and each 3 samples of menstrual blood and vaginal fluid. Methylation values are reported as betas (fraction between 0 and 1), which represent the methylation level of each CpG site. Based on delta mean value (difference in average beta for each body fluid), we selected body fluid-specific candidate methylation CpG markers. To identify CpG loci for which the methylation values significantly correlated with age, we performed Spearman correlation and linear regression analysis. Clustering analysis of selected candidate CpG markers was performed with BDPC program (http://services.ibc.uni-stuttgart.de/BDPC/).

Methylation SNaPshot

To validate the reliability of the selected CpG site from microarray data, we performed gene specific analysis in a larger group of samples (49 males and 16 females) using methylation SNaPshot method. One to ten nanogram of bisulfite-converted DNA was amplified in a 20 µL final volume containing 1.0 U of AmpliTaq Gold® DNA Polymerase (Applied Biosystems), 2.0 µL of Gold ST*R 10× Buffer (Promega, Madison, WI, USA), and appropriate concentrations of each primer. Thermal cycling was conducted under the following conditions: 95˚C for 11 min; 34 cycles of 94˚C for 20 sec, 56˚C for 1 min, 72˚C for 30 sec; and a final extension at 72˚C for 7 min. For the following single-base extension (SBE), 5.0 µL of PCR product was purified with 1.0 µL of ExoSAP-IT (USB, Cleveland, OH, USA). SBE reaction was carried out with a SNaPshot™ Multiplex Kit (Applied Biosystems) according to the manufacturer's instructions. Extension products were analyzed using a 3130 Genetic Analyzer (Applied Biosystems) and GeneMapper® ID software v.3.2 (Applied Biosystems).

Results

Fig. 1. Clustering analysis of 66 body fluid-specific candidate CpG markers (A) Methylation map of 66 selected CpG sites in 42 body fluid samples. (B) Similarity plot based on Pearson's correlation coefficient for each sample pairs. (C) Hierarchical clustering of 42 samples with the 66 candidate CpG markers.

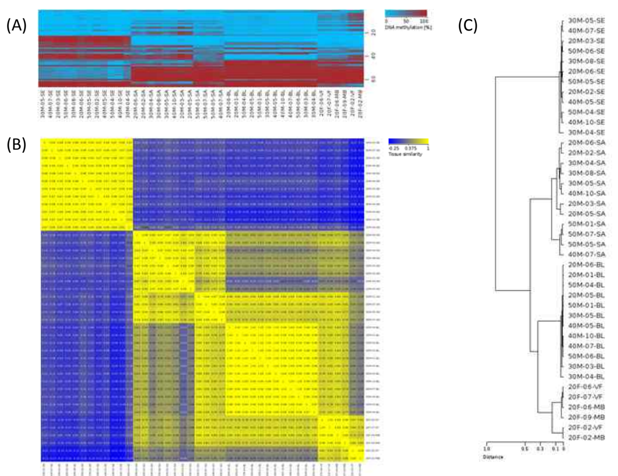


Fig. 2. Representative electropherograms of methylation SNaPshot results of body fluid-specific candidate CpG markers (A) A blood specific marker. (B) A saliva specific marker. (C) A menstrual blood-vaginal fluid specific marker. (D) A semen specific marker.

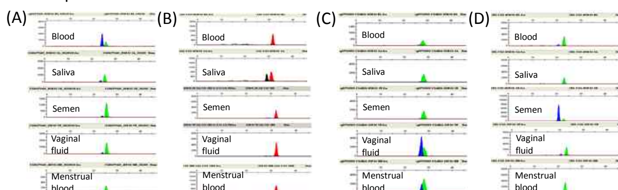


Table 1. Candidate CpG markers correlated with age Candidate CpGs were selected with R^2 of more than 0.7 in linear regression analysis, variation in DNA methylation level between young and elderly donors (Δβ) as larger than 20% and Spearman correlation *p*-values (*p*) of smaller than 0.05.

Body fluids	Cut-off	Result CpG	Sig. Result CpG*
BLOOD	$r^2>0.7$ & $\Delta\beta>10\%$, $p<0.05$	477,632	**110**
SALIVA	$r^2>0.7$ & $\Delta\beta>20\%$, $p<0.05$	478,955	**170**
SEMEN	$r^2>0.7$ & $\Delta\beta>20\%$, $p<0.05$	479,686	**96**

*Sig. Result CpG : the number of CpG sites which meet the cut-off criteria

Fig. 3. Clustering analysis of age-associated candidate CpG markers (A) 110 selected age-related CpG sites in blood samples. (B) 170 selected age-related CpG sites in saliva samples. (C) 96 selected age-related CpG sites in semen samples.

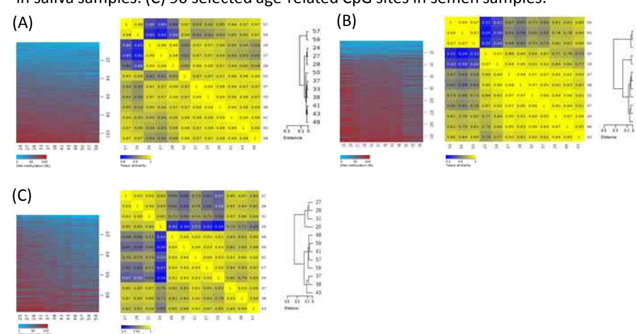
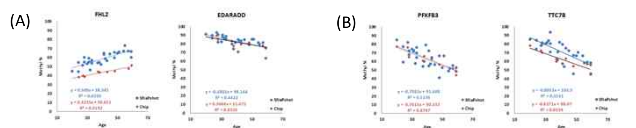


Fig. 4. Age-associated methylation changes Methylation level of the selected CpG markers are plotted against donor age. (A) Blood age-related markers. (B) Semen age-related markers.



Conclusion

- We analyzed DNA methylation profile of 42 body fluid samples including venous blood, saliva, semen, menstrual blood and vaginal fluid using the Illumina Infinium Human Methylation450 BeadChip array and selected 66 body fluid-specific and 376 age-associated (110 for blood, 170 for saliva and 96 for semen) candidate CpG markers.
- Through the further validation test using Methylation SNaPshot, we confirmed that a subset of CpG markers showed more strong correlation with body fluid origin or age and could be proposed as useful biomarkers in forensic sciences.

Acknowledgement

- This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (2012R1A1A2007031) and a grant from the National Forensic Service funded by the Ministry of Public Administration and Security (No. 1315000435).