DNA methylation markers as a powerful technique to discriminate body fluids present in crime scenes

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INTRODUCTION

DNA analysis in forensics allows us to determine if a suspect was present or not at a given crime scene. In forensic cases where the presence of a suspect's DNA is expected at the crime scene, it is often probative to know if the fluid is saliva or semen, for example; however, current tests for body fluid identification are merely presumptive and lack sensitivity and specificity. The detection of saliva currently depends on the enzymatic activity of amylase in saliva using the Phadebas test [1]. Amylase is present in serum, urine, the pancreas and some plants and bacteria, making this detection merely presumptive. The identification of sperm relies on the detection of acid phosphatase and prostate-specific antigen, both produced in the prostate gland. [2] These tests rely on the presence of a native protein and can therefore be subject to degradation or inhibitory effects from the surrounding environment. Recently the possibility of discriminating body fluids using mRNA expression differences in tissues has been described in the literature [3-8] but lack of long term stability of that molecule and the need to normalize samples for each individual are limiting factors. The use of DNA should solve these issues because of its long-term stability and specificity to each body fluid. Cells in the human body have a unique epigenome [9], which includes differences in DNA methylation in the promoter of genes. The presence or absence of a methyl group on the 5' carbon of the cytosine pyridine ring in CpG dinucleotide regions called 'CpG islands' [10] dictates whether the gene is expressed or silenced in the particular body fluid. Eckhardt et al. [11] described methylation patterns at tissue-specific differentially methylated regions (tDMRs) to be stable and specific, making them excellent markers for tissue identification. The goal of this project was to identify genetic markers that are indicative of a blood, saliva, semen and epithelial cells.

METHODS

Whole genomic DNA extracted using the Phenol-Chloroform method is bisulfite modified using the Epitect Bisulfite conversion kit (Qiagen, CA) in order to convert each unmethylated cytosine to an uracil, while keeping the methylated cytosines. Samples are amplified by PCR using primers specifically designed to anneal the bisulfite converted DNA (Pyromark PCR kit, Qiagen, CA). The fragment is analyzed by pyrosequencing to determine the percent of methylation at each variable position. Four different locations on the genome were determined to discriminate blood, saliva and semen. Initial tests used 10 blood, 11 saliva, 10 semen and 11 epithelial cells to determine the specificity of the markers. Five blood samples stored at room temperature for 9 years in FTA® paper four blood and 1 semen samples stored for 20 years in fabric, paper towel and regular paper were also tested to determine if the pattern of methylation remains constant during long term storage. Three mixtures containing different ratios of blood and semen were tested to determine the capacity of distinguishing mixtures. DNA from several non-human species were tested to determine species-specificity of the markers. At University of Southern Mississippi new genome locations were tested using between 3 and 5 samples of the following body fluids: blood, saliva, semen, skin epithelials and vaginal epithelials. Two genome locations were identified as potential markers for semen discrimination.

RESULTS

Differential DNA methylation at 4 different locations allows the discrimination of 3 different body fluids

The C20orf117 allows the discrimination of blood when compared to the other body fluids tested since all 7 CpGs within the location present higher levels of methylation for blood (Figure 1A). BCAS4 genome location shows higher levels of methylation for saliva compared to blood, semen and skin epithelial cells (Figure 1B). The markers FGF7 and ZC3H12D allow the discrimination of semen. FGF7 is hypermethylated (Figure 1C) in semen when compared to other body fluids tested whereas in ZC3H12D all semen samples are hypomethylated with values around 10% while other body fluids present methylation levels around 100% (Figure 1D).

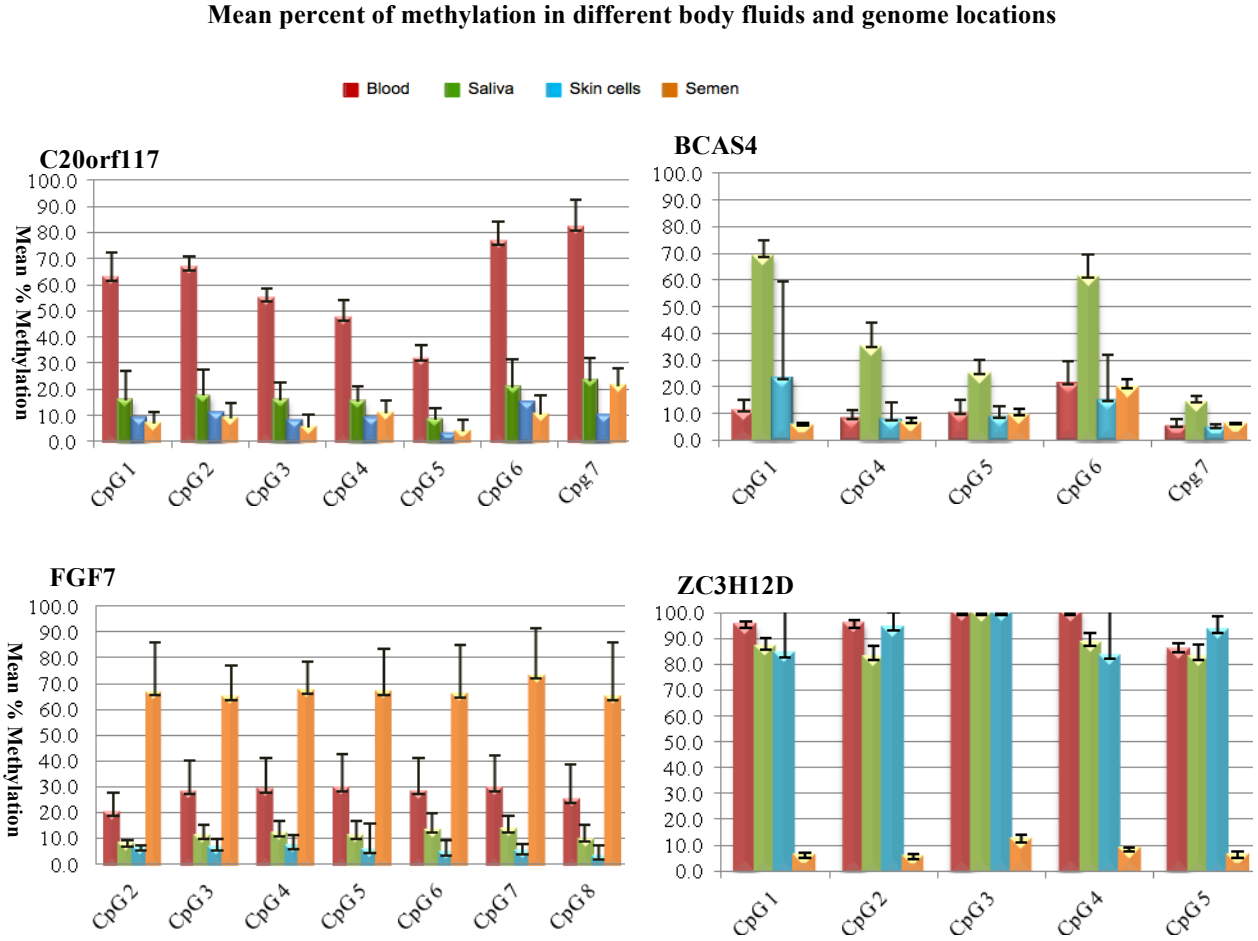

Figure 1 –In C20orf117 blood data is mean for 9 samples; saliva, 11 samples; epithelial cells from skin, 1 sample; semen 9 samples. In BCAS4 blood data is mean for 10 samples; saliva, 11 samples; epithelial cells from skin, 9 samples; semen 10 samples. In FGF7 blood is mean for 10 samples; saliva, 10 samples; epithelial cells from skin, 8 samples; semen, 9 samples. In ZC3H12D blood is mean for 10 samples; saliva, 11 samples; epithelial cells from skin, 8 samples; semen, 10 samples. Error bars are standard deviation from the mean. [12]

Methylation levels are constant for long-term stored samples when compared to samples collected recently

Blood samples collected 9 years before the date of study in FTA® paper were analyzed for their mean percent of methylation. In all 4 markers the five samples of blood in FTA® paper have similar values of methylation as the samples recently collected. Blood and semen samples collected 20 years before time of study also present the same methylation pattern when compared to samples collected recently.

Different ratios of blood and semen present intermediate percent of methylation

When mixture samples with different ratios of blood and semen DNA are analyzed they present intermediate methylation values compared to the blood only and semen only. The correlation between the methylation values are as expected where mean methylation drops as the content in blood DNA decreases.

Markers tested for non-human DNA show species specificity

Samples from non-human species were tested in parallel with DNA from human to serve as a positive internal control. Most of the samples failed to amplify not showing any band in the 2% agarose gel. For chicken and one bacterium (*Pseudomonas spp*.) there were several visible bands although none corresponded to the size expected for that marker and observed in the human DNA positive control. When those samples were analyzed by pyrosequencing none showed peaks on the pyrogram.

New genome locations identified to be used as semen markers

Two new locations on the genome were identified as semen discrimination markers since both show hypomethylation for semen and hypermethylation for the other body fluids tested.

CONCLUSION

Knowing with certainty the body fluid from where a DNA sample was extracted constitutes important information for crime scenes where the presence of the suspect is expected. The natural occurring variability in methylation content on DNA allows us to identify body fluids from crime scenes. Using bisulfite modification and pyrosequencing we were able to determine 6 genome locations capable of discriminating 3 body fluids. The methylation of DNA proved to be stable for samples stored up to 20 years and tests of species specificity demonstrate no cross reactivity with non-human DNA. Need the results here…

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