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Examination of Methylation Sites for Forensic Age Determination from Semen

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Introduction

- Age determination is critical to forensic sexual assault investigations; adequate age estimation would assist investigators with a correct identification
- Age-related changes in cytosine methylation (C → 5mC) at certain loci have been reported from blood and saliva in several studies
- Common methylation analysis involves beadchip assays and pyrosequencing. Few forensic laboratories are equipped with these instruments and high costs prevents routine usage
- Methylation-sensitive high resolution melting (MS-HRM) measures methylation status easily and cost effectively, using bisulfite-treated and PCR amplified DNA
- Previous MS-HRM study has shown methylation at the ELOVL2 and FHL2 CpG islands in blood samples directly relate to age

Methods

Extraction
- Semen samples from 7 individuals were extracted using QIAamp DNA Investigator Kit

Bisulfite Conversion
- Bisulfite conversion of extracted DNA was conducted using EpiTect Bisulfite Kit

PCR amplification and High Resolution Melt Analysis
- Amplification was conducted using QuantStudio 6 Flex Real-Time PCR System
- DNA standards of known methylation values were co-analyzed: 0, 25%, 50%, 75%, 100%
- Melt Curve analysis was conducted using EpiTect HRM PCR Kit

Standard Curve Modeling and Methylation Prediction
- Df value was assigned to absolute minimum value on difference plot with 100% methylation standard set as baseline
- Df values of DNA standards were used to generate reference standard curve

ELOVL2 Standards Melt Curve

Non-linear Regression with Df Values as a Function of Percent Methylation

\[ y = d + \frac{a - d}{1 + \left( \frac{x}{c} \right)^b} \]

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>ELOVL2</th>
<th>FHL2</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>2.95347</td>
<td>3.22090</td>
</tr>
<tr>
<td>b</td>
<td>6.5962</td>
<td>9.55867</td>
</tr>
<tr>
<td>c</td>
<td>6.51756</td>
<td>3.24281</td>
</tr>
<tr>
<td>d</td>
<td>13495287.1</td>
<td>4656593.3</td>
</tr>
<tr>
<td>x/% Methylation</td>
<td>% Methylation</td>
<td></td>
</tr>
<tr>
<td>y/Df Value</td>
<td>Df Value</td>
<td></td>
</tr>
</tbody>
</table>

Results

- Methylation values calculated as negative percentages using the standard curve regression were categorized as “Unknown”
- Minimal resolution for methylation values below 50% inhibited accurate quantification

Conclusions

- Results indicate that no correlation may exist between age and methylation status at these loci in semen
- Small sample size prohibits extrapolation to population

Potential Future direction

- Expand analysis of ELOVL2 and FHL2 loci to other forensically relevant body fluids; evaluate potential variability in methylation differences across male and female samples
- Evaluate ELOVL2 and FHL2 loci in blood to confirm published results
- Resources permitting, conduct an exploratory Whole-Genome Sequencing analysis for semen to identify semen-specific loci related to age

Acknowledgements

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