Analysing DNA methylation levels of the age predictor gene EDARADD using the bisulfite PCR-sequencing method

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Introduction

Adult’s age-at-death estimation is one of the main concerns in the forensic field. Recently, DNA methylation of some genes emerges as a powerful tool in age-at-death estimation [1]. A correlation between DNA methylation status of the EDARADD (EDAR associated death domain) gene (on chromosome 1q42.3) and chronological age of individuals was previously shown [2, 3]. In this study, we investigated the correlation between DNA methylation patterns of four CpGs from EDARADD gene and chronological age in a Portuguese sample.

Methods

Blood samples of 47 healthy individuals (32 females, 15 males; aged 1-95 years old) were collected after informed consent and according institutional and ethical guidelines. Genomic DNA was extracted and a method based on the bisulfite conversion using EZ DNA Methylation-Gold™ Kit (Zymo Research, CA, USA), followed by PCR and Sanger sequencing was used to evaluate the DNA methylation patterns in four CpGs of EDARADD gene. The methylation status of cytosines in each CpG dinucleotide was estimated by measuring the ratio of the cytosine peak height to the sum of cytosine and thymine peak heights, according to [4]. Simple linear regressions were used to analyze relationships between the CpGs methylation levels and chronological age. Statistical analysis was performed using SPSS, version 24.0.

Results and discussion

Table 1: Univariate regression analysis of 4 CpGs of EDARADD gene.

<table>
<thead>
<tr>
<th>CpG</th>
<th>R</th>
<th>Corrected R²</th>
<th>Standard error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>0.046</td>
<td>-0.020</td>
<td>31.066</td>
<td>0.759</td>
</tr>
<tr>
<td>C2</td>
<td>0.797</td>
<td>0.627</td>
<td>18.783</td>
<td>2.05×10⁻¹¹</td>
</tr>
<tr>
<td>C3*</td>
<td>0.912</td>
<td>0.828</td>
<td>12.745</td>
<td>4.75×10⁻¹⁹*</td>
</tr>
<tr>
<td>C4*</td>
<td>0.610</td>
<td>0.358</td>
<td>24.649</td>
<td>0.0000005</td>
</tr>
</tbody>
</table>

*Investigated according to the literature.

A negative correlation between EDARADD DNA methylation levels and chronological age was observed (Figure 1). A strong correlation between methylation levels and age was observed for C3 (R = 0.912; p = 4.75×10⁻¹⁹), explaining 83% of variation in age, followed by C2 (R = 0.797; p = 2.05×10⁻¹¹) (adjusted R² = 0.627). The C4 site showed a lower correlation (R = 0.610; p = 0.000005) (adjusted R² = 0.358) and C1 revealed no significant correlation with age (R = 0.046; p = 0.759). Data showed in table 1.

Figure 1: Negative correlation between DNA methylation levels from CpG3 of EDARADD gene and chronological age (years).

Figure 2: Predicted age versus chronological age of the 47 individuals based in C3 methylation levels.

Conclusion

Bisulfite PCR-sequencing showed to be a simple, efficient and economic method to investigate DNA methylation levels at EDARADD gene. C3 showed to be an accurate age predictor site and C2 a site that should be considered in future age estimation models.

References