Evaluating the DNA methylation patterns of *ELOVL2* gene based on the bisulfite PCR-sequencing: a useful method for human age prediction

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Introduction

Adult's age-at-death estimation is one of the main concerns in the forensic field for human identification. Methods routinely used are mainly based on dental development and bone degenerative changes. However, the anthropological approaches only produce estimations with a relative large range in advanced ages [1,2]. In addition, there are also several biomolecular and chemical methods that seem to be able to achieve better accuracy in age estimation but, at the same time, have other inconsistencies [3]. Recently, DNA methylation of some genes emerged as a powerful tool of Forensic Genetics in many contexts, including age-at-death estimation [4]. It has been shown that DNA methylation patterns are preserved in ancient samples and can be effectively used to infer chronological age [5,6]. Some studies have shown a strong correlation between DNA methylation status of the *ELOVL2* (*fatty acid elongase 2*) gene on 6p24.2 and chronological age of individuals [7,8,9,10]. *ELOVL2* methylation status has already been investigated in several tissues and in bloodstains based on bisulfite conversion followed by pyrosequencing, allowing good accuracy of age estimation [7,8,9]. In this study, we investigated the correlation between DNA methylation patterns of 9 CpGs from *ELOVL2* gene and chronological age in a sample of Portuguese subjects based on bisulfite PCR-sequencing, a method that has not been used until now for human age estimations.

Methods

Blood samples of 48 healthy individuals (32 females, 16 males; aged 1-95 years old) were collected after informed consent and according institutional and ethical guidelines. Genomic DNA was extracted using a commercial kit and subjected to bisulfite conversion using the *EZ DNA Methylation-Gold™ Kit (Zymo Research, CA, USA)*. PCR amplification of *ELOVL2* gene fragment was performed using primers previously designed [8], followed by *Sanger* sequencing. The methylation status of cytosines in each CpG dinucleotides was estimated by measuring the ratio of the cytosine peak height to the sum of cytosine and thymine peak heights (C/C+T), according to [11]. Simple linear regressions were used to analyze relationships between the CpGs methylation levels and chronological age. Statistical analysis was performed using SPSS software, version 24.0.

Results and discussion

A positive correlation between *ELOVL2* DNA methylation and chronological age was observed for all examined CpGs (data showed for CpG6 in figure 1).

Simple linear regression testing the association between methylation levels and age revealed strong correlations for all CpGs (R > 0.80) (Table 1). The strongest correlation was observed for C6 (R = 0.943; p = 1.50×10^{-23}), explaining 88.6% of variation in age, followed by C5 (R = 0.937; p = 1.24×10^{-22}) (adjusted R^2 = 0.875) and C7 (R = 0.932; p = 6.10×10^{-22}) (adjusted R^2 = 0.866).
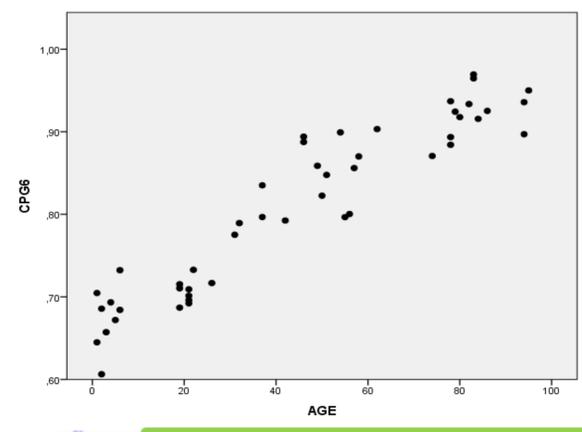
Figure 1: Positive correlation between CpG6 DNA methylation levels and chronological age.

Table 1: Univariate regression analysis of the 9 CpG sites in *ELOVL2* locus.

CpG	R	Corrected R^2	Standard error	P value
C1	0.921	0.846	11.96	1.65×10^{-20}
C2	0.869	0.751	15.21	1.10×10^{-15}
C3	0.881	0.772	14.56	1.44×10^{-16}
C4	0.921	0.844	12.02	2.04×10^{-20}
C5	**0.937**	0.875	10.76	**1.24×10^{-22}**
C6	**0.943**	0.886	10.28	**1.50×10^{-23}**
C7	**0.932**	0.866	11.13	**6.10×10^{-22}**
C8	0.848	0.713	16.30	2.74×10^{-14}
C9	0.859	0.732	15.77	5.96×10^{-15}

Figure 2: Plot with predicted age versus chronological age of the 48 individuals based on the C6 methylation levels.

Predicted age of the sampled individuals based on the strongest correlation site C6, showed a mean absolute deviation from chronological age of 7.82 years. For two individuals aged 1 and 2 years old, negative prediction values were obtained and were set at 0. A plot with predicted age (years) versus chronological age (years) is presented in figure 2.
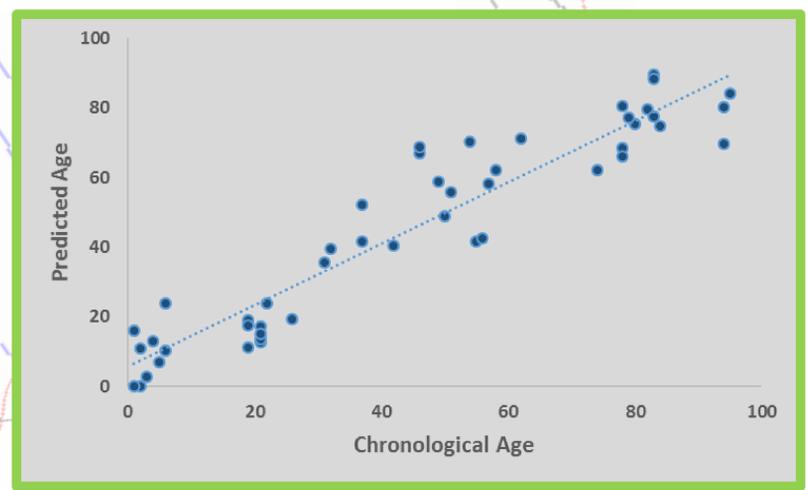
Conclusion

The bisulfite PCR-sequencing showed to be a simple, efficient and economic method for quantification of DNA methylation patterns in *ELOVL2*, a powerful age predictor in concordance with previous studies. This method consisting of bisulfite conversion followed by PCR and *Sanger* sequencing allows accuracy similar to the commonly used method pyrosequencing and can be considered as a basis for future age estimation models.

Sponsors

References

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