Bisulfite PCR-sequencing as a method to evaluate the DNA methylation patterns of the age predictor gene *ELOVL2*

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Introduction

In forensic investigations, age estimation has a crucial role, complementing the prediction of externally visible characteristics. Recently, DNA methylation of some genes emerges as a powerful tool of Forensic Genetics in many contexts, including age-at-death estimation [1]. Some studies have shown a strong correlation between DNA methylation status of the *ELOVL2* (*fatty acid elongase 2*) gene (6p24.2) and chronological age of individuals [2,3,4,5,6,7]. *ELOVL2* seems a good age predictor and has been investigated in several tissues [8] and in bloodstains [6]. In this study, we have investigated the correlation between DNA methylation patterns of 9 CpGs from *ELOVL2* and chronological age based on the bisulfite PCR-sequencing method, which has not been used until now for human age estimations.

Methodology

Blood samples of 48 Portuguese healthy subjects (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 [4], 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 [9]. Simple linear regressions were used to analyze relationships between each CpG methylation level and chronological age. Statistical analysis was performed using SPSS software, version 24.0.

Results and discussion

Figure 1 shows the different patterns of DNA methylation of 5 analyzed CpGs from the *ELOVL2* gene in two individuals with different ages. Higher levels of methylation can be observed in the older in relation to the younger subject.

A positive correlation between *ELOVL2* DNA methylation and chronological age was observed for all examined CpGs. Figure 2 shows the correlation between DNA methylation levels in C6 versus chronological age.

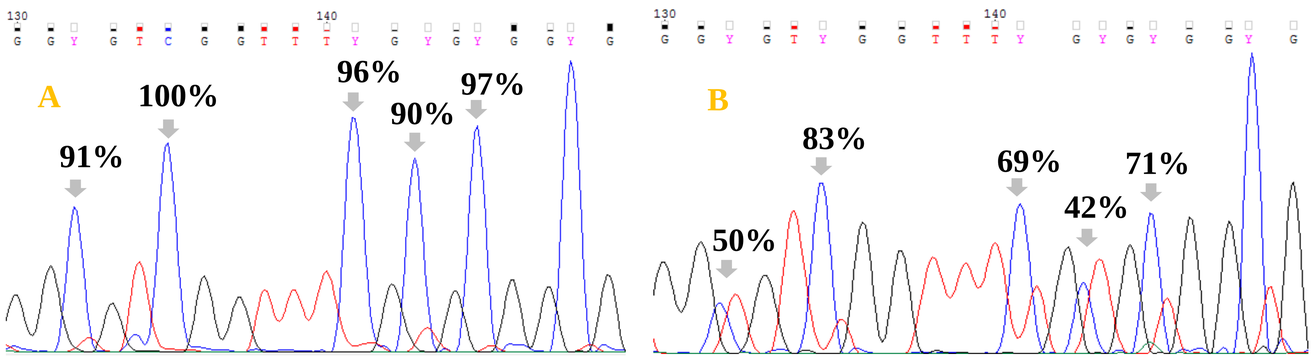

Figure 1: DNA methylation levels of *ELOVL2* locus in individuals with 83 years old (A) and 26 years old (B).

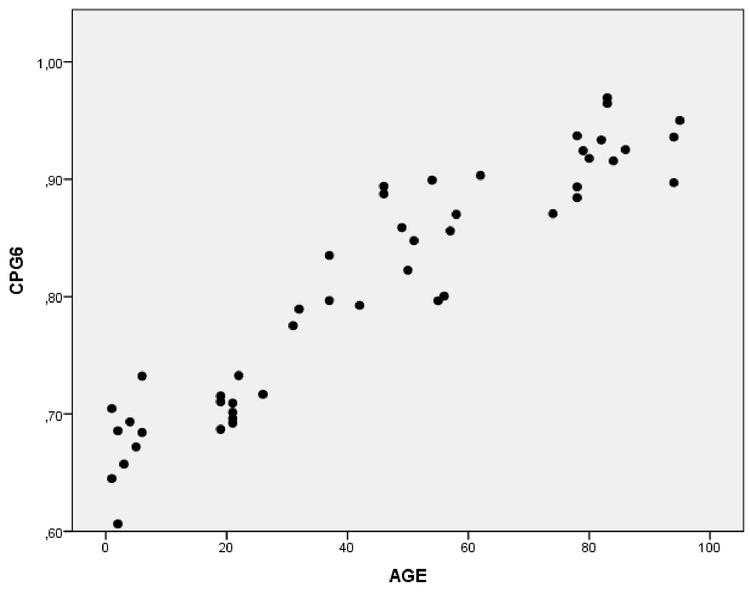

Figure 2: Positive correlation between CpG6 DNA methylation levels and chronological age.

Simple linear regression testing the association between *ELOVL2* DNA methylation levels and chronological age revealed strong correlations for all CpGs ($R > 0.80$).

The strongest correlation was observed for C6 ($R = 0.943$; $p = 1.50\times10^{-23}$), explaining 88.6% of variation in age (adjusted $R^2 = 0.886$), followed by C5 ($R = 0.937$; $p = 1.24\times10^{-22}$) (adjusted $R^2 = 0.875$) and C7 ($R = 0.932$; $p = 6.10\times10^{-22}$) (adjusted $R^2 = 0.866$) (Table 1).

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	$\mathbf{1.24\times10^{-22}}$
C6	**0.943**	0.886	10.28	$\mathbf{1.50\times10^{-23}}$
C7	**0.932**	0.866	11.13	$\mathbf{6.10\times10^{-22}}$
C8	0.848	0.713	16.30	2.74×10^{-14}
C9	0.859	0.732	15.77	5.96×10^{-15}

Conclusion

In conclusion, the bisulfite PCR-sequencing method showed to be an 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, can be considered as a basis for future age estimation models.

References

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