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SHORT TAKE

Methylation of *ELOVL2* gene as a new epigenetic marker of age

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Summary

The discovery of biomarkers able to predict biological age of individuals is a crucial goal in aging research. Recently, researchers' attention has turn toward epigenetic markers of aging. Using the Illumina *Infinium* HumanMethylation450 BeadChip on whole blood DNA from a small cohort of 64 subjects of different ages, we identified 3 regions, the CpG islands of *ELOVL2*, *FHL2,* and *PENK* genes, whose methylation level strongly correlates with age. These results were confirmed by the Sequenom's EpiTYPER assay on a larger cohort of 501 subjects from 9 to 99 years, including 7 cord blood samples. Among the 3 genes, *ELOVL2* shows a progressive increase in methylation that begins since the very first stage of life (Spearman's correlation coefficient = 0.92) and appears to be a very promising biomarker of aging.

Key words: aging; biomarker; DNA methylation; *ELOVL2*; *FHL2*; *PENK*.

Aging is a complex process characterized by a global decline in physiological functions and is associated with an increased risk for several diseases. A great effort is made to find reliable biomarkers of aging and epigenetic represents one of the most promising fields (Berdasco & Esteller, 2012). Epigenome-wide association studies (EWAS) report that CpG island, mainly placed within genes promoter regions, are hypermethylated in the elderly (Bell *et al.*, 2012). In this study, we analyzed the whole blood DNA methylation profile of 32 mother–offspring couples using Illumina *Infinium* HumanMethylation450 BeadChip. The age range was 42–83 and 9–52 years for mothers and offspring, respectively. ANOVA analysis identified 163 CpG sites differentially methylated between these two groups. The top 5 significant loci mapped within the CpG islands of *ELOVL2*, *FHL2* and *PENK* genes, and other loci within *ELOVL2* and *PENK* had a *P-value* below the Bonferroni threshold (Fig. 1A,B). The CpG islands of *ELOVL2*, *FHL2,* and *PENK* – all located in the respective gene promoter – resulted hypermethylated in mothers compared with offspring (Fig. 1C), with no sex or family-associated bias. Spearman's correlation analysis for the 3 genes showed striking correlation values between methylation levels and age of the subjects.

We replicated these results in a larger sex-balanced cohort using Sequenom's EpiTYPER assay. The analyzed samples included whole blood DNA from 494 individuals (245 men and 249 women) ranging from 9 to 99 years, plus 7 cord-blood DNA samples (3 males and 4 females). Depending on the sequence, the EpiTYPER assay returns the methylation values of single CpGs or small groups of close CpGs (CpG units). Samples were divided in 5 age classes, whose mean methylation values for each CpG unit are reported in Fig. 2A. We then calculated Spearman correlation between age and methylation level for each CpG unit. The highest correlation values obtained were 0.92 (CpG_11.12.13.14), 0.80 (CpG_9.10 and CpG_19.20), and 0.63 (CpG_23.24) for *ELOVL2*, *FHL2,* and *PENK,* respectively (Fig. 2B). In all cases, the considered sites tended to be hypermethylated with advancing age (Fig. 2C, for *FHL2* only the CpG_9.10 is shown). *ELOVL2* displayed the widest methylation range, from 7% to 91% (for FHL2 and PENK, they were 12% to 53% and 1% to 27%, respectively, Fig. 2C).

In each gene, we identified a subset of CpG units, which displayed high coefficients of correlation with age and whose methylation values were closely correlated with each other (Fig. 2D). The subset of highly correlated CpG units was used to perform principal components analysis (PCA). The first principal component (PC1) was calculated, and boxplot distributions of PC1 values in the 10 decades of age (Fig. 2E) showed an increase in methylation level of the considered regions.

In this study, we identified and validated 3 genes, *ELOVL2*, *FHL2,* and *PENK*, whose CpG islands methylation changes with age. *FHL2* and *PENK* showed very high correlation values, but with a small difference between the different age classes. At variance, *ELOVL2* displayed not only striking correlation levels, but also an almost 'on-off' methylation trend between the two extremes of life, ranging from 7% to 91% of methylation.

The hypermethylation of CpG islands during aging is well described (Bell *et al.*, 2012), and several DNA methylation biomarkers displaying a good correlation with age have been described (Bocklandt *et al.*, 2011). In our study, taking advantage of a more dense DNA methylation microarray technology, we identified and validated more striking and reproducible age biomarkers. To date, the lack of highly reproducible aging biomarkers is explained by the high levels of heterogeneity of aging phenotypes (Cevenini *et al.*, 2008), and only within this framework it is possible to appreciate the extraordinary, progressive hypermethylation of *ELOVL2* that continuously increases from the very first stage of life to nonagenarians. We cannot exclude that this is due to numerical alterations of specific blood cell subpopulations, and further studies are needed to clarify this issue. Nevertheless, it is worth noting

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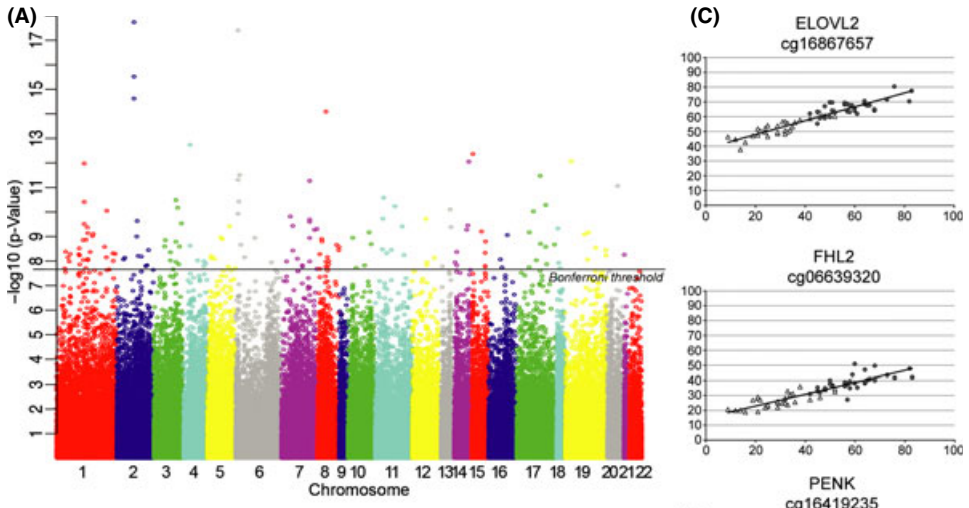

(B)

Rank position	Target ID	Gene name	*p*-Value	Correlation with age
1	cg06639320	FHL2	1.82E-18	0.91
2	cg16867657	ELOVL2	3.97E-18	0.91
3	cg22454769	FHL2	3.00E-16	0.92
4	cg24079702	FHL2	2.37E-15	0.86
5	cg16419235	PENK	8.10E-15	0.76
13	cg21572722	ELOVL2	4.92E-12	0.89
26	cg24724428	ELOVL2	1.16E-10	0.85
129	cg16219603	PENK	1.05E-08	0.62
149	cg12877723	PENK	1.65E-08	0.63

Fig. 1 EWAS study. (A) Manhattan plot of genome-wide *P*-values from mother–offspring comparison. (B) *P*-values and Spearman correlation values of the selected CpG sites in *ELOVL2*, *FHL2,* and *PENK* CpG islands. The *P*-value of each probe was ranked from the smallest to the largest, and the position of the selected probes in the list is reported in the first column. (C) Methylation of the selected CpG sites respect to age. Circles: mothers; triangles: offspring.

that in previous studies of gene expression in T cells, correlations with gene expression patterns and changes of T subpopulations with age were not observed (Remondini *et al.*, 2010).

ELOVL2 encodes for a transmembrane protein involved in the synthesis of long (C22 and C24) ω3 and ω6 polyunsaturated fatty acids (PUFA) (Leonard *et al.*, 2002), and it is mainly expressed in the liver, while its expression and role in human blood cells have not been properly addressed. Genome-wide studies identified *ELOVL2* genetic variants associated with serum metabolic profile, especially with the serum concentration of specific n-3 PUFAs (Tanaka *et al.*, 2009; Lemaitre *et al.*, 2011). To date, *ELOVL2* has not been associated with aging. In light of our results, and considering that PUFAs are involved in crucial biological functions including energy production, modulation of inflammation, and maintenance of cell membrane integrity, it is possible that *ELOVL2* methylation plays a role in the aging process through the regulation of different biological pathways.

The outstanding results obtained on *ELOVL2* make it a strong candidate for forensic applications aimed at identifying proband age. To this purposes, other tissues, such as saliva and hair, should be investigated. Secondly, *ELOVL2* age-dependent hypermethylation is also a promising candidate as biomarker for the evaluation of individual fitness in elder people, with the potential for early diagnosis of age-related diseases or for monitoring therapeutic intervention or disease course.

In conclusion, (i) DNA methylation of CpG islands of *ELOVL2*, *FHL2,* and *PENK* shows strong correlation with age and, in particular *ELOVL2* is the most extreme example of age-related hypermethylation, constituting a bridge between the first developmental stages and the aging process. (ii) *ELOVL2* could be used in forensic sciences and in clinical applications. (iii) Finally, *ELOVL2* could be proposed as a sort of rheostat for aging – the more is methylated, the more aged is the subject. Further studies are needed to understand whether *ELOVL2* hypermethylation only represents an indicator of chronological age or rather is functionally correlated with physiological status and specific clinical conditions.

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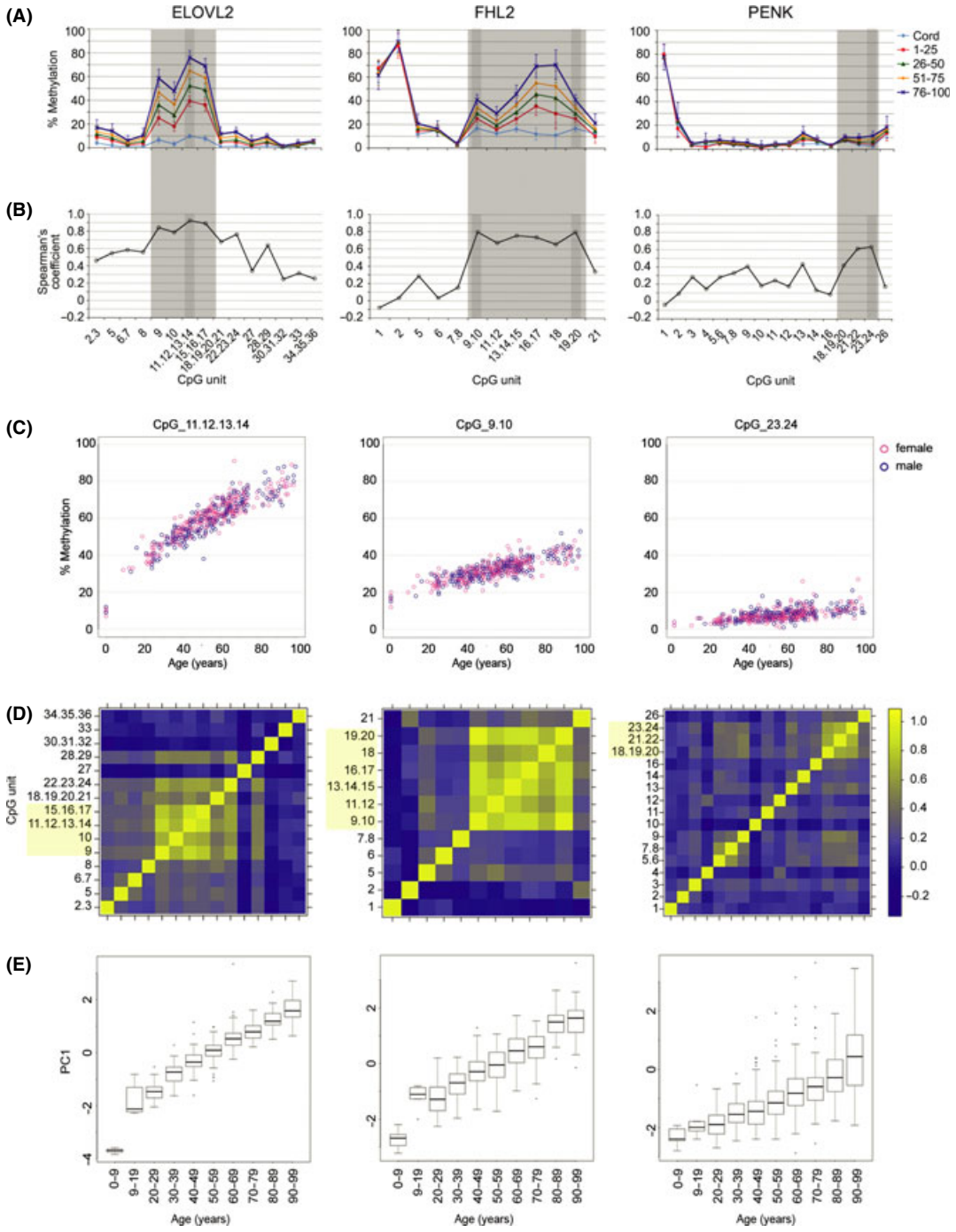

Fig. 2 Replication study. (A) Mean methylation values ± standard deviation in 5 age classes are reported for each CpG unit. (B) Spearman's correlation coefficients for each CpG unit. Highly correlated regions are marked in gray. (C) Methylation values of the CpG unit that better correlates with age in each gene. (D) Correlation between CpG units within each CpG island. The most correlated CpG units are highlighted in yellow. (E) Distribution of PC1 values calculated in 10 age classes.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Data S1. Experimental procedures.