SHORT TAKE

Methylation of ELOVL2 gene as a new epigenetic marker of age

Paolo Garagnani,1,2 Maria G. Bacalini,1,2 Chiara Pirazzini,1,2 Davide Gori,3 Cristina Giuliani,4 Daniela Mari,5,6 Anna M. Di Blasio,7 Davide Gentilini,7 Giovanni Vitale,5,7 Sebastiano Collino,8 Serge Rezzi,8 Gastone Castellani,9 Miriam Capri,1,2 Stefano Salvioni1,2 and Claudio Franceschi1,2

1Department of Experimental Pathology, University of Bologna, Bologna, Italy
2Interdept. Centre for Bioinformatics, Biophysics and Biocomplexity L. Galvani, University of Bologna, Bologna, Italy
3Department of Medicine and Public Health, University of Bologna, Bologna, Italy
4Department of Experimental Evolutionary Biology, University of Bologna, Bologna, Italy
5Department of Medical Sciences, University of Milan, Milan, Italy
6Geriatric Unit IRCSS Cà Grande Foundation Maggiore Policlinico Hospital, Milan, Italy
7Istituto Auxologico Italiano, Milan, Italy
8Metabolomics and Biomarkers, Department of BioAnalytical Science, Nestlé Research Center, Nestec Ltd., Lausanne, Switzerland
9Physics and Biophysics, Department of Physics, University of Bologna, Bologna, Italy

Summary

The discovery of biomarkers able to predict biological age of individuals is a crucial goal in aging research. Recently, researchers’ attention has turned 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...
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) α3 and α6 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 hypermethylation 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.

Acknowledgments

This research has received funding from the European Union’s Seventh Framework Programme (grant agreement no. 259679, “IDEAL”).

References


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.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Experimental procedures.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.