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Aging and may predict onset of Alzheimer disease up to 10 years before the clinical diagnosis (Boraxbekk et al., 2015; Jack and Holtzman, 2013). However, the impact of aging, to a degree where functions are impaired, varies markedly across individuals, and the underlying biological mechanism for such variability remains poorly understood (Lopez-Otin et al., 2013). Epigenetic alterations during aging refer to modifications of DNA and histone proteins that directly influence chromatin structure and thereby gene expression and genomic stability (Jones, 2007; Lin and Wagner, 2015; McCabe et al., 2009). These modifications are partly reversible and result from stochastic events as well as environmental factors. DNA can be methylated on cytosine bases, mostly adjacent to guanine bases, known as CpG sites (Jones, 2012). Changed CpG site methylation profiles have been associated with altered physical and cognitive function as well as development and progression of cancer (Klein et al., 2016; Levine et al., 2015; Lin and Wagner, 2015; Lin et al., 2016; Marioni et al., 2015b; McCabe et al., 2009). Moreover, aging-associated changes in DNA-methylation (DNAm) on specific genomic positions have been shown to correlate well with chronological age, a phenomenon that denoted the epigenetic clock (Hannum et al., 2013; Horvath, 2013; Horvath et al., 2015a; Lin et al., 2016). Accelerated epigenetic aging has been associated with all-cause mortality later in life and physical and cognitive fitness (Chen et al., 2016; Horvath, 2013; Horvath et al., 2015a; Lin et al., 2016). The potential reversibility of epigenetic changes may entail opportunities to alter the trajectory of age-related syndromes.

Here, we examined epigenetic age in 3 subgroups classified by differences in longitudinal episodic-memory outcome.
2. Materials and methods

The participants originated from the Betula longitudinal cohort study on memory, health, and aging in Sweden, initiated in 1988 (Nilsson et al., 2004). The individuals were previously classified as having maintained high episodic memory performance (“Maintainers”), average decline (“Averages”), or accelerated decline (“Decliners”) based on their performance on repeated episodic memory tests over 15–20 years. Individuals were classified as having episodic memory decline/maintenance if their rates of change fell below/above 1 standard deviation of a model prediction that accounted for their age and individual baseline performance, and corrected for dropout (Fig. 1A, Supplementary Materials and Methods) (Josefsson et al., 2012; Pudas et al., 2013).

Age- and sex-matched subjects, n = 16 from the Decliners group, n = 16 from the Maintainers, and n = 20 from Averages, met the inclusion criteria for this study. Exclusion criteria were prior/current cancer chemotherapy. Blood samples from the selected 52 individuals were analyzed by HumMeth450K arrays at baseline (55–65 years of age) and 15 years later. Three additional replicate samples were included, which showed high concordance (R^2 = 0.994–0.997). All study samples (N_{tot} = 104), except 2, passed the built-in quality control in the arrays. The 2 failed samples and the replicate samples were excluded, and 102 samples remained for further analyses.

The age of an individual can be estimated by DNA methylation analysis on a tissue sample, and we used the 353 CpG sites “epigenetic clock” prediction model described by Horvath to determine the biological epigenetic (DNAm) age of the blood samples (Horvath, 2013). This model has been shown to predict chronological age ±3.6 years with a good correspondence between blood and other tissue samples, which provides a solid basis for analysis of epigenetic age in blood and relate results to brain functions (Horvath, 2013). Linear mixed models were used to assess the longitudinal association between epigenetic DNAm age and the memory groups. The models included a random intercept for subject and for matched group. Logistic regression models were used to assess the association between age and occurrence of dementia, after controlling for gender. The dirichlet regression analysis was used to compare the distribution of estimated leukocyte cell

![Fig. 1.](image)

(A) Mean episodic memory scores at 5-year intervals across memory groups classified as having maintained high performance (Maintainers), having average decline (Averages), or having accelerated decline (Decliners) over 15–20 years. Baseline (T2) and 15-year follow-up (T5) are marked in the figure. Error bars represents ±1 standard error. (B) Mean delta DNAm age over 15 years based on Horvath model across the memory groups and (C) individuals’ delta DNAm age at baseline and at 15-year follow-up. In the respective memory groups, plain lines: individuals’ 15-year trajectories of delta DNAm age and dashed lines: mean delta DNAm age. Abbreviation: DNAm, DNA-methylation.
subsets between the memory groups. Linear mixed models and the logistic regression models were run in R using the libraries “lme4” and “ImeRlest”, and the “glm” function in the “stats” library. Detailed description of the study cohort, cognitive and dementia classification, and methods are available as Supplementary Data.

3. Results

A delta DNAm age was calculated by subtracting the predicted DNAm age from each individual’s chronological age at sampling, allowing samples of slightly different age at analysis to be compared. The memory groups’ delta DNAm age was compared at the group and individual levels (Fig. 1 and Table 1). At the group level, agreement between DNAm age (mean 57.1 years) and chronological age (mean 57.9 years) was observed (cor = 0.69, p < 0.001) at baseline (T2) but with a disparity at follow-up (T5, 70–80 years of age) when the majority of individuals were predicted younger than their chronological age (mean 69.5 respectively 72.8; data not shown, Fig. 1).

In the longitudinal group analyses, predicted DNAm age differed significantly among the memory groups. The Maintainers group showed a significant 2.7, respectively 2.8 years younger predicted DNAm age over the study period compared with Averages and Decliners (p = 0.035 respectively p = 0.037) (Fig. 1B, Table 1). There were interindividual variations within the groups, but in the Maintainer group, most individuals were predicted younger than their chronological age, both at baseline, mean: −2.6 years and p = 0.018, and at 15 years later, mean: −5.1 years and p < 0.001 (Fig. 1C). There was no significant difference in the DNAm aging rates (i.e., the DNAm age-slope: 0.79–0.81) during the 15-year period between the groups, but interindividual differences were observed (Table 1, Fig. 1B).

There was a marked difference in frequency of dementia progression (vascular dementia or Alzheimer’s disease) at or after the follow-up time point across the memory groups (maintainer, n = 0/16; average, n = 2/20; and decliner, n = 9/16; Table 1). Additional analyses of the association between occurrence of dementia and age at follow-up were done by specifying separate logistic regression models to predict the occurrence of dementia (epigenetic predictor for occurrence of dementia (beta = 0.16, p = 0.019), whereas chronological age was not (beta = 0.12, p = 0.268). Similar, but weaker results were obtained using baseline age (epigenetic age at baseline: beta = 0.12, p = 0.087, chronological age at baseline: beta = 0.12, p = 0.263). Crucially, DNAm age at follow-up predicted dementia significantly even after controlling for chronological age (beta = 0.16, p = 0.032).

To assess potential influence of risk factors for episodic memory decline on DNAm age, we included the following lifestyle, health, and genetic characteristics (Josefsson et al., 2012) in a multivariate analysis: years of education, labor force participation, whether the participant was living with someone, smoking habits, and APOE/COMT genotype (Supplementary Table S1). No significant effects (p > 0.05) of the covariates on DNAm age were found when using mixed models, neither when the complete set of covariates were included nor when the covariates were included one-by-one.

It is known that epigenetic pattern could differ between leukocyte subsets and that leukocyte subset composition changes by age (Cheng et al., 2004; Reinius et al., 2012). The epigenetic age prediction model by Horvath has been constructed to work on different cell types. Here, we used data for cell composition collected at baseline to rule out that our results reflect cell subset variations rather than difference in epigenetic cellular age. We found no significant difference in total white blood cells, neutrophils, eosinophils, basophils, or lymphocytes concentration at baseline (Supplementary Table S2) between the memory groups. No monitoring of complete blood cell counts with differential counts was performed at follow-up. To overcome this, we used the prediction model of leukocyte subset distribution based on epigenetic profiling (Acomando et al., 2014) both at baseline and at follow-up. We observed a reduced proportion of CD4+ cells and increased proportion of monocytes and granulocytes over the 15-year follow-up period (Supplementary Fig. S1A). These age-associated changes in white blood cell composition have been described in the literature (Carr et al., 2016; Cheng et al., 2004).

There was a strong concordance of the measured proportions and epigenetically estimated proportions of leukocyte blood cell subsets at baseline [granulocytes: p < 0.001, lymphocytes: p < 0.001, and monocytes: p = 0.019] (Supplementary Fig. S1B). Importantly, there were no significant differences between the memory groups in estimated leukocyte subset distribution during aging that could have biased the results (Supplementary Fig. S1).

4. Discussion

Most previous studies on DNA methylation and cognition used cross-sectional designs. The strength of this study is the longitudinal design with dynamic analyses of epigenetic aging in peripheral blood collected 15 years apart. Our findings suggest that younger epigenetic age at the age of 55–80 years may be a potential mechanism contributing to preserved episodic memory functioning in adulthood and aging, and potentially also contribute to reduced risk of dementia.

The biology behind the finding of younger predicted DNAm age in the Maintainer group remains to be determined. However, the

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td>Study population demographic, cognitive, and epigenetic data across memory groups</td>
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<table>
<thead>
<tr>
<th>Memory class</th>
<th>Maintainer</th>
<th>Average</th>
<th>Decliner</th>
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<tr>
<td>Number of individuals</td>
<td>16</td>
<td>20</td>
<td>16</td>
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<tr>
<td>Mean age at baseline T2-follow-up T5 (SD)</td>
<td>57.8 (3.6)/72.8 (3.5)</td>
<td>58.0 (3.5)/72.8 (3.5)</td>
<td>57.9 (3.6)/72.7 (3.6)</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>8/8</td>
<td>9/11</td>
<td>8/8</td>
</tr>
<tr>
<td>Mean first memory score (SD)</td>
<td>45.9 (6.0)</td>
<td>36.5 (5.5)</td>
<td>29.0 (6.1)</td>
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<tr>
<td>Memory slope</td>
<td>−0.07 (0.44)</td>
<td>−0.17 (0.37)</td>
<td>−0.73 (0.64)</td>
</tr>
<tr>
<td>Mean MMSE score baseline T2-follow-up T5 (SD)</td>
<td>27.8 (1.6)/28.1 (1.1)</td>
<td>28.5 (2.1)/27.7 (2.3)</td>
<td>27.4 (1.5)/26.1 (3.3)</td>
</tr>
<tr>
<td>Dementia (AD or VaD)</td>
<td>0</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Mean epigenetic age (SD) baseline T2-follow-up T5 (SD)</td>
<td>54.7 (4.7)/67.6 (6.2)</td>
<td>58.2 (6.2)/70.2 (6.3)</td>
<td>58.1 (5.8)/70.4 (7.4)</td>
</tr>
<tr>
<td>Mean epigenetic delta age (SD) baseline T2-follow-up T5 (SD)</td>
<td>−2.61 (3.26)/−5.11 (4.48)</td>
<td>0.20 (4.53)/−2.61 (4.63)</td>
<td>−0.00 (4.31)/−2.28 (7.41)</td>
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<tr>
<td>Mean epigenetic aging (SD) T5 (SD)</td>
<td>0.81 (0.31)</td>
<td>0.81 (0.20)</td>
<td>0.79 (0.32)</td>
</tr>
</tbody>
</table>

Key: AD, Alzheimer’s dementia; SD, standard deviation; VaD, vascular dementia.

* Mini Mental State Examination.

b Horvath predicted age.
results are in line with previous, typically cross-sectional, studies showing associations of accelerating DNAm age and impaired cognitive functions, posttraumatic stress, poor working memory, and even shortened life expectancy (Chen et al., 2016; Lin and Wagner, 2015; Marioni et al., 2015a,b; Wolf et al., 2016). Forthcoming large-scale analyses will further determine whether epigenetic markers of peripheral tissues as blood can be used as a proxy for changes occurring in the brain. In a recent report, concordant and discordant DNA methylation signatures were identified in matched samples of human blood and brain (Farre et al., 2015).

Separate analyses revealed that epigenetic age at follow-up was a significant predictor for occurrence of dementia, whereas chronological age was not. Future larger studies are needed to correlate DNAm age with dementia progression with higher statistical power, but tentatively, the present findings that maintained memory is predicted by young epigenetic age may be extended to lowered risk for dementia progression.

Health- and lifestyle-related factors may also influence epigenetic age and memory ability in aging. Such factors may explain a selective epigenetic advantage for the maintainer group relative to the decliners and average, as well as the large inter-individual variability in predicted epigenetic age observed even in the decline group. We found no significant association between DNAm age and known risk factors for episodic memory decline, but the sample size was limited, and these factors need to be further analyzed in larger studies to better explicate the causal pathways between epigenetic modifications, lifestyle, and genetic factors that previously have been linked to maintained memory functioning in aging (Josefsson et al., 2012; Nyberg et al., 2012).

The majority of individuals were predicted epigenetically younger than their chronological age at follow-up (at age 70–80), in contrast to at baseline (at age 55–65). The discrepancy might indicate that the Horvath age prediction model slightly underestimates the ages in older individuals, which was also observed in recent studies on the Lothian Birth Cohort 1936 and centenarians, respectively (Horvath et al., 2015b; Marioni et al., 2016). However, this systematic deviation does not affect our main finding since the Maintainer group was predicted epigenetically younger than the Average and Maintainer cognitive group both at baseline and at 15-year follow-up. In addition, it may reflect a selection effect of the entire cohort such that older participants coming for follow-up assessments presumably are healthier than the general population (Cooney et al., 1988).

5. Conclusions

In summary, we use a longitudinal design to study epigenetic age dynamics in groups with different memory trajectories over a 15-year period. Our findings suggest that younger epigenetic age at the age of 55–80 years may be a potential mechanism contributing to preserved episodic memory functioning in adulthood and aging, and potentially also contribute to reduced risk of dementia. Future studies of epigenetic alterations in episodic memory decline and dementia are of importance to evaluate the potential of epigenetics as an alternative therapeutic target.

Disclosure statement

The authors declare no conflicts of interests to disclose.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2017.02.009.

References


Levine, M.E., Lu, A.T., Bennett, D.A., Horvath, S., 2015. Epigenetic age of the prefrontal cortex is associated with neuropsychic plaques, amyloid load, and


