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Early View

Research letter

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Epigenetic Age Acceleration in Obstructive Sleep Apnea is Reversible with Adherent Treatment

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Conflicts of Interest

The authors declare no conflict of interest.

Dear Editor,

Obstructive Sleep Apnea (OSA) leads to activation and propagation of oxidative stress and systemic inflammatory pathways, essentially mimicking accelerated biological aging (senescence) [1–3]. Biological aging is a complex and time-dependent deterioration of physiological processes with attendant morbidity and mortality. During aging there is continuous and accelerated accumulation of epigenetic changes manifesting either systemically or restricted to a specific tissue/cell type. *Epigenetic Clocks* or *DNA Methylation Clocks*, have emerged as valuable biological age prediction tools[4]. By regressing DNA methylation age on chronological age, epigenetic clocks can determine whether biological age acceleration occurs in certain diseases or in response to environmental factors [4]. Using this approach, age acceleration measurements in blood were abnormally high in the context of common conditions such as obesity, neurological diseases, and cigarette smoking [5].

We hypothesized that patients with OSA will present systemic epigenetic age acceleration compared to controls. We further posited that treatment of OSA with adherent continuous positive airway pressure (CPAP) would lead to deceleration of the epigenetically-based biological aging, whereas no changes in baseline measurements would occur in untreated controls.

We studied a set of individuals enrolled in the EPIOSA study (NCT02131610). Details on patient recruitment are reported elsewhere[6] . Age range of participants was 28–58 years, all individuals were non-smokers, and underwent an overnight in-lab polysomnographic evaluation. Patients with polysomnographically diagnosed OSA at baseline and after 12 months of adherent CPAP treatment were selected (n=16). The adequate adherence criterion to CPAP was >4 hours/night. Matched non-snoring controls, whose overnight polysomnography (PSG) was normal (apnea-hypopnea index (AHI) < 5 events/hour sleep), were enrolled and reevaluated after 12 months (n = 8). Data from all sleep studies were scored using AASM guidelines by

trained personnel that were blinded to the aims or nature of the study. Adherence to CPAP therapy was measured using the machines' internal timers. Each participant was evaluated during the initial visit (V1) and a year later (V2), during which fasting blood samples after overnight PSG were collected and peripheral blood mononuclear cells (PBMC) were separated and stored at - 80 °C until use. Laboratory analyses (e.g., CRP, Total cholesterol, HDL and LDL) were conducted as described elsewhere[6]. DNA methylation profiles were analyzed using Illumina's Infinium Human Methylation 450 BeadChip assay (Illumina, San Diego, CA). The dataset is available at the NCBI Gene Expression Omnibus (GEO) repository (accession number pending). All data analyses were conducted using the R environment version 4.1.0. Microarray data was processed using the *minfi* package version 1.38.0. DNA methylation clocks were derived according to the method developed by Hannum and colleagues [7]. Principal component analysis and variable plots were conducted using the *factoextra* package version 1.0.7.

OSA and control groups were matched by ethnicity, chronological age, and sex, had similar blood pressure, and slight differences in BMI, with OSA subjects displaying significantly higher AHI and high-sensitivity CRP levels (Figure 1A). All OSA patients were treated with CPAP and displayed excellent adherence (average CPAP use: 6.03 □□0.81 hours/night). Blood cell composition inferred from the DNA methylation profiles was not significantly different between OSA and Control individuals at V1 and V2 nor between V1 and V2 for OSA and Control individuals ($p>0.05$; Student's T-test).

Epigenetic clocks were determined from the DNA methylation profiles of PBMC from OSA subjects and controls. Epigenetic age variation from the chronological age was assessed for each study participant at V1 and V2 (Figure 1B). Principal Component Analysis (PCA; Figure 1C) showed that OSA patients clustered separately from controls. In turn, post-CPAP (V2) samples for OSA patients migrated and clustered closer to the controls, while no changes

occurred over time in controls. Graph of variables (Figure 1D) revealed the differential acceleration residuals between visits V1 and V2 (AccResV2.V1) was the variable dragging the sample distribution towards the control samples. Conversely, increases in parameters related to poor cardiovascular outcome (i.e., IMT, plaques, SBP and DBP) and OSA-related risk factors (i.e., AHI and BMI) dragged sample distribution towards the position of OSA samples. AccResV2.V1 was the output of the epigenetic clocks that best discriminated between OSA and controls (Figure 1E). In contrast, estimates of DNA methylation age (DNAmAge) and chronological age discriminated the groups to a much lesser extent. Noteworthy, similar PCA results were obtained when a different epigenetic clock estimate, i.e., DNAmGrimAge[8], was applied (Data not shown).

Whereas controls retained increased epigenetic age acceleration in the year between the first and second visits (mean AccResV2.V1=1.41 $\pm$ 1.96: Figure 1F), OSA patients showed a significant reduction in the epigenetic acceleration metric between the two visits (mean AccResV2.V1=-1.03 $\pm$ 0.48; OSA *vs*. Controls: p-value=5.5 $x10^{-4}$; F-test). Furthermore, the percentages of variation in the mean AccResV2.V1 were 62% and 5% in the Control and OSA groups, respectively, (Figure 1G). Epigenetic age deceleration observed in OSA patients may be ascribed to CPAP treatment. Noteworthy, second visit samples are more closely clustered with controls in OSA patients with lower CRP levels (0.02-0.28 mg/dL) than in patients with higher CRP levels (0.4-0.94 mg/dL) (Figure 1H), suggesting that deceleration in epigenetic aging observed in OSA patients receiving CPAP treatment is attenuated in those patients with increased inflammation. None of the other clinical variables registered in these individuals (i.e., BMI, SBP, DBP, and cholesterol, HDL, and LDL levels) significantly correlated with the acceleration of epigenetic age ($p>0.05$, Pearson's correlation test).

In summary, OSA patients displayed a higher acceleration of the systemic epigenetic age when compared with controls, and adherent treatment with CPAP for 12 months using therapeutic

pressures that normalize respiratory and sleep patterns resulted in a deceleration of the epigenetic age, whereas the epigenetic age acceleration trends remained unaltered in the control subjects. A previous work reported that severe SDB was associated with epigenetic age acceleration [9], yet the impact of CPAP therapy on aging and cellular senescence has been scarcely investigated. Yagihara and colleagues reported that patients with severe OSA had a younger appearance following a month of CPAP treatment compared with those receiving placebo[10]. However, the perceived age in this correlation study was arbitrarily determined, with no precise assessments of biological age. On the other hand, it has been shown that CPAP treatment improves markers of age-associated OSA morbidities[1, 2], such as cognitive impairment, vascular dysfunction, nocturnal polyuria, elevated systolic and diastolic blood pressure, and gait impairment. Remarkably, CPAP treatment increases the blood concentration and activity of Sirtuin 1 (SIRT1), a histone/protein deacetylase with reduced expression in aging and cellular senescence [11]. Furthermore, CPAP-treated OSA patients also showed increased levels of Nitric Oxide derivatives (NOx), which are products of endothelial nitric oxide synthase (eNOS), a SIRT1-regulated enzyme [11]. In earlier studies, we uncovered initial evidence of accelerated vascular senescence and cellular aging induced by OSA [12], which may be initiated or exacerbated either directly or indirectly via exosomes [13],

Our results suggest that OSA-induced perturbations promote biological age acceleration, and that such processes are at least partially reversible when adherent and effective treatment of OSA is implemented. Although the cellular and molecular mechanisms underlying the accelerated biological senescence are still unclear, our study provides a novel framework for the management of adult OSA and its associated morbidities, whereby evaluation of patient variability in epigenetic age acceleration may open new opportunities for molecular diagnostics and personalizing clinical management.

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FIGURE LEGEND

Figure 1: Assessment of epigenetic age acceleration in OSA patients and controls. A) Demographic characteristics of OSA and Control groups. B) Epigenetic age estimated using the DNAmAge models correlates with biological age in OSA and control samples at V1 and V2. C) PCA plot using all outputs of Epigenetic clocks variables and co-morbidities distinguishes between OSA patients as well as controls (red and blue shapes, respectively). D) Graphic of variables indicating the direction and contribution for each variable in the PCA analysis from panel C. Positively and negatively correlated variables point to the same or opposite side of the plot, respectively. The contributions to the sample discrimination are color coded in a gradient from red (higher contribution) over yellow to light blue (lower contribution). E) PCA plot using only AccResV2.V1 as output of the epigenetic clock. OSA and control samples are identified as in Panel C. F) Mean values for the difference in age acceleration residuals between visit 1 and visit 2 (AccResV2.V1) in OSA (red bar) and controls (blue bar). Differences between the groups are statistically significant (p-value=5.5 $x10^{-4}$; F-test). Error bars correspond to Standard Error of the Mean (SEM). G) Percentage of variation in mean age acceleration residuals between visit 1 and visit 2 (AccResV2.V1) in OSA (red bars) and controls (blue bars). H) Assessment of epigenetic age acceleration in OSA patients stratified by CRP levels. PCA plots using only AccResV2.V1 as output of the epigenetic clock for OSA patients with high and low CRP levels (left and right panels, respectively). Second visit (V2) samples clustered more closely to controls in patients with low CRP OSA patients (right panel) than in high CRP OSA patients (left) suggesting an attenuated epigenetic age deceleration in patients with high inflammation. Red and pink shapes correspond to high CRP OSA samples in V1 and V2, respectively. Dark and light brown shapes correspond to low CRP OSA samples in V1 and V2, respectively. Blue and light blue shapes correspond to control samples in V1 and V2, respectively.

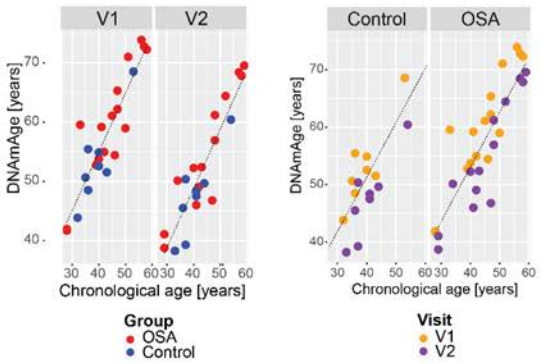
Figure 1

A

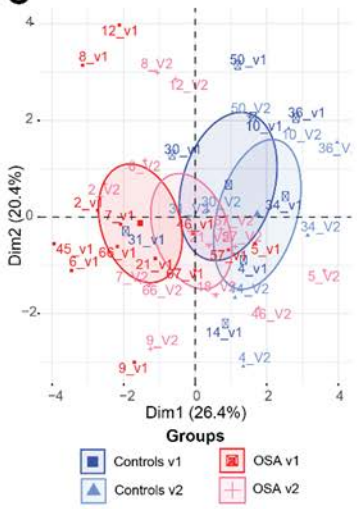
		OSA	Controls	p-value*
n		16	8	
Ethnicity		16/16 Caucasian	8/8 Caucasian	
*Sex***		16/16 male	7/8 male	
Chronological age	*Visit 1*	44.3 ± 9.3 years	39.4 ± 6.5 years	0.153
	Visit 2	45.3 ± 9.6 years	40.4 ± 6.5 years	0.135
Body Mass Index (BMI)	*Visit 1*	31.6 ± 3.9 kg/m²	26.8 ± 2.5 kg/m²	**0.002**
	Visit 2	31.4 ± 4.0 kg/m²	26.5 ± 3.0 kg/m²	**0.004**
Systolic Blood Pressure (SBP)	*Visit 1*	131.7 ± 16.0 mmHg	125.5 ± 14.2 mmHg	0.344
	Visit 2	130.9 ± 13.0 mmHg	117.5 ± 11.6 mmHg	**0.023**
Diastolic Blood Pressure (DBP)	*Visit 1*	82.6 ± 9.7 mmHg	77.0 ± 12.2 mmHg	0.280
	Visit 2	78.4 ± 13.2 mmHg	72.8 ± 8.8 mmHg	0.236
Apnea Hypopnea Index (AHI)	*Visit 1*	67.2 ± 20.3 events/hour	2.5 ± 2.4 events/hour	**1.2×10^{-9}**
	Visit 2	6.8 ± 12.5 events/hour	2.0 ± 0.9 events/hour	0.145
Total cholesterol level	*Visit 1*	230.6 ± 54.1 mg/dL	212.0 ± 42.1 mg/dL	0.367
	Visit 2	219.9 ± 55.6 mg/dL	204.8 ± 45.4 mg/dL	0.490
High-density lipoprotein (HDL) level	*Visit 1*	45.3 ± 7.5 mg/dL	52.1 ± 10.6 mg/dL	0.135
	Visit 2	45.1 ± 8.3 mg/dL	48.4 ± 9.1 mg/dL	0.407
High-density lipoprotein (LDL) level	*Visit 1*	148.1 ± 44.6 mg/dL	142.8 ± 41.4 mg/dL	0.779
	Visit 2	144.7 ± 45.5 mg/dL	133.3 ± 39.4 mg/dL	0.540
Intima media thickness (IMT)	*Visit 1*	0.62 ± 0.10 mm	0.54 ± 0.10 mm	0.081
	Visit 2	0.67 ± 0.21 mm	0.64 ± 0.18 mm	0.708
C- Reactive Protein (CRP)	*Visit 1*	0.35 ± 0.25 mm	0.08 ± 0.09 mm	**0.001**
	Visit 2	0.34 ± 0.21 mm	0.20 ± 0.23 mm	0.193

*: Student's t-test, significant differences (p<0.05) are in bold.
**: The inclusion of the female control did not modify the main findings of the study (Supplementary Figure S1).

B



C All variables Epigenetic Clock variables



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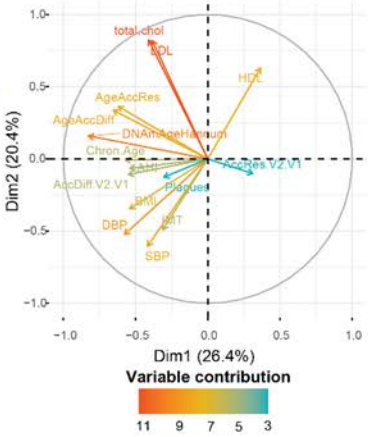

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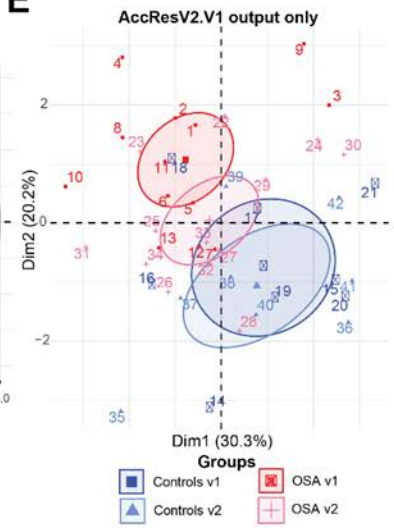

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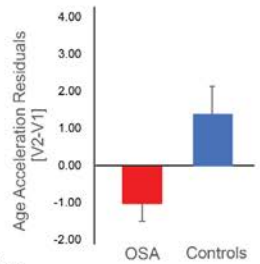

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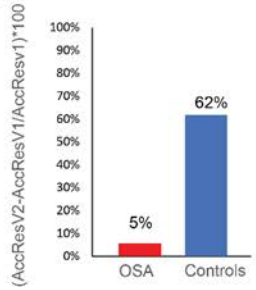
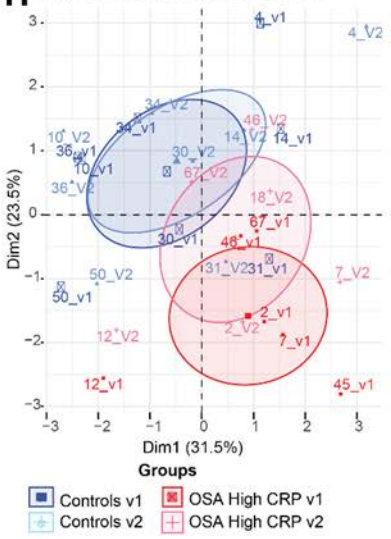


H High CRP OSA patients and controls



Low CRP OSA patients and controls