La Medicina del Lavoro

Med Lav 2021; 112, 1: 8-14
DOI: 10.23749/mdl.v112i1.10826

Is this environment making you older? Molecular biomarkers and new approaches to investigate the influences of environmental chemicals through aging

Diddier Prada[1], Dan Belsky[2], Andrea A. Baccarelli[3]

[1] Mailman School of Public Health, Columbia University, USA; Instituto Nacional de Cancerología, Mexico
[2] Department of Epidemiology, Mailman School of Public Health, Columbia University, USA; Robert N Butler Columbia Aging Center, Columbia University, USA
[3] Laboratory of Precision Environmental Health, Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, USA

Keywords: Environment; epigenetics; age acceleration; biological clocks, DNA methylation; epitranscriptomics; aging

Abstract

Aging is characterized by a gradual and progressive decline in system integrity that occurs with advancing chronological age. Although it is a physiological process, aging is associated with a myriad of age-related diseases (ARDs), including frailty, sarcopenia, chronic obstructive pulmonary disease, cardiovascular disease, cancer, and neurodegenerative diseases. While not exclusively ARDs, many of these diseases lead to death, a lesser quality of life, and increased healthcare costs for individuals and systems. ARDs share several underlying molecular mechanisms, such as cellular damage, inflammation, DNA methylation changes, stem cells exhaustion, and DNA mutations, which have been outlined as hallmarks of aging. Evidence suggests that environmental exposures, including but not limited to metals, air pollution, endocrine-disrupting chemicals, and noise, may accelerate biological aging. Over the past few years, aging research has identified new molecular biomarkers of the aging process. When applied to investigate environmental influences, these biomarkers can help identify individuals who are particularly susceptible to the influences of environmental exposures on aging processes and therefore guide in implementing possible preventive measures.

Introduction

Aging is characterized by a gradual and progressive decline in system integrity that occurs with advancing chronological age (1, 2). The physiological changes that occur with aging contribute to a myriad of age-related syndromes including frailty and sarcopenia, and diseases (ARDs) such as chronic obstructive pulmonary, cardiovascular, cancer, and neurodegenerative diseases (3). These conditions account for a substantial population burden of disability and premature mortality. ARDs share several molecular mechanisms, including cellular damage (4), inflammation (5), epigenetic alterations (6), stem cells exhaustion (7), and DNA mutations (8), outlined as hallmarks of aging (9). Environmental exposures, including metals, air pollution, endocrine-disrupting chemicals, and noise, may affect these hallmarks, accelerating biological processes and leading to a reduction in lifespan, a

Received 30.10.2020 - Accepted 12.11.2020
Andrea Baccarelli, Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, USA
email: andrea.baccarelli@columbia.edu

phenomenon called 'age acceleration.' While aging hallmarks themselves are challenging to measure in-vivo, researchers have recently identified several biomarkers thought to reflect the accumulation of changes in the hallmarks. These biomarkers measure the process of biological aging and can be applied to investigate the impacts of environmental exposures. Key applications include the detection of harmful effects, identifying individuals who are most susceptible or most severely affected, and evaluating the effectiveness of mitigation efforts, and therefore, guiding the implementation of possible preventive measures.

Environment and aging

Available evidence suggests that all these *hallmarks of aging* are interrelated. External factors such as exposures to environmental chemicals, as well as behaviors (e.g., unhealthy diet and smoking), modify theses molecular mechanisms, accelerate aging, and predispose to ARDs (10). It has been estimated that environmental causes impact up to 90% of human diseases (11). Air pollution is one of the most pervasive of these causes and accelerate ARDs such as cardiovascular and lung disease, bone damage (12, 13), cognitive decline (14), and cancer (15). Air pollution exposure modeling, which integrates data from different sources, including stationary air pollution monitors, point sources, traffic data, and satellite measures of aerosol optical density (e.g., MODerate resolution Imaging Spectroradiometer, MODIS) (16), has been applied to facilitate the determination of exposure in populations at risk. These technologies have helped us to refine effect estimates on human health. In a nationwide analysis of the United States between 1980 and 2010, hybrid approaches (i.e., land-use regression, traffic indicators, and Bayesian maximum entropy interpolation of land-use regression space-time residuals) were used to determine the levels of particulate matter < 2.5 μm ($PM_{2.5}$) in 28 million adults from 3,034 counties, as well as the effect of the exposure and other common factors on exceptional aging (defined as reaching 85 years of age). We found that communities with the most exceptional aging showed low ambient air pollution and low rates of smoking, poverty, and obesity (17). These findings suggest several avenues of study to explore mechanisms by which environmentally-related factors may modify underlying biological mechanisms of aging, as well as the potential for this information to be used to reduce ARDs and promote healthy aging. To realize this potential, biomarkers are needed that can identify individuals with accelerated biological aging attributable to environmental exposures.

Epigenetics as a target and a biomarker of environmental-related damage

Our team has been working with epigenetic modifications as an interface between the environment and human health. DNA methylation (DNAm) is one of the most well-studied epigenetic markers. It plays a critical role in several cellular processes, including gene regulation, imprinting, X-chromosome and retrotransposon silencing, and chromosome stability (18). DNAm is a chemical modification that occurs at carbon five in the CpG context (cytosine-phosphate-guanine) in mammalian organisms. Using some of the platforms showed in **Box 1**, we have demonstrated that air pollution may modify peripheral blood DNA methylation, affecting, among others, genes involved in mitochondrial oxidative energy metabolism (19), inflammation (i.e., ICAM-1) (20), and mitogen-activated protein kinase (MAPK) network (21). However, air pollution is not the only environmental exposure that may affect DNA methylation. Other studies have shown that pesticides (22), metals (23) and several other environmental toxicants may modify the epigenome (24). Machine learning methods now make it possible to use whole-genome DNAm data to model and measure exposure to environmental

Box 1. Epigenome-wide approaches to study DNA methylation in human population studies:1

- Illumina's Infinium BeadChips (450K/850K methylation CpG sites).
- Targeted methylation sequencing (3.3/5.5M methylation CpG sites).
- Whole-genome bisulfite sequencing (28M methylation CpG sites)

toxicants. Our group recently used this approach to develop a fingerprint biomarker of chronic lead exposure (23). We have also constructed a minimally invasive lead biomarker based on 59 and 138 peripheral blood CpG sites, which could be used to reconstruct decades' worth of individual cumulative lead exposure. Remarkably, this same approach can be expanded to other environmental exposures. Ultimately, these DNAm models of toxicant exposure can catalyze development a new generation of environmental risk assessments. Because these models are all based on the same platform, a single assay will enable measurement of exposure to multiple different toxicants.

Environmentally induced damage and acceleration of biological aging

Human (and in general mammalian) chronological aging brings with biological changes that drive decline in system integrity, increasing risk for disease, disability, and mortality (25). Measures that quantify processes of biological aging—sometimes called biological clocks—include telomere length (26), transcriptomic age (27, 28) glycan age (29), among others (30), can detect differences in aging-related risks among individuals who are the same chronological age (31), suggesting that chronological age does not accurately reflect biological age, which may proceed at a different rate (32). The accurate determination of biological age and age acceleration using biological clocks offers the opportunity for early prevention of onset and progression of chronic diseases. Among these measures, DNA methylation clocks, particularly those described separately by Hannum (33) and Horvath (34), are among the most studied at present. These clocks are derived from machine learning analysis applied to epigenome-wide DNA methylation data. Clock-ages that are older than chronological ones indicate advanced biological aging and have been linked with increased risk for morbidity and mortality (35). Our team has recently demonstrated that clock ages are advanced in individuals with higher levels of exposure to environmental toxicants, as we found with exposure to particulate air pollution which resulted associated with age acceleration—measured through telomere shortening or accelerated DNA methylation age—in peripheral blood in older adults (36-38). We have also found that this difference between chronological age and DNA methylation age (Δ_{age}) may predict mortality by any cause in older individuals (39), suggesting that individuals with higher levels of age acceleration will show early aging and early death (39). Additionally, we have reported that air pollution, in particular long-term $PM_{2.5}$, sulfate, and ammonium, are linked with particular changes in DNA methylation age (particularly sulfate and ammonium) (40). Evidence surrounding the role of other environmental factors that modify biological age and induce age acceleration is also available for metals (e.g., cadmium) and pesticides (e.g., organochlorides) (41).

DNA methylation changes can also be used to predict future risk of ARDs. A seminal work from our group evaluated more than 11,000+ individuals from longitudinal studies and found that methylation levels at 52 CpG sites are associated with two established ARDs: incident coronary heart disease (CHD) and myocardial infarction (42). These CpGs are related to calcium regulation (*ATP2B2, CASR, GUCA1B, HPCAL1*), serum calcium (*CASR*), serum calcium-related risk of CHD (*CASR*), coronary artery calcified plaque (*PTPRN2*), and kidney function (*CDH23, HPCAL1*), among others. These data suggest the possibility that blood DNA methylation may contribute to build-up predictive tools that forecast future development of ARDs (42). DNA methylation has also been associated with several other ARDs and age-related risk factors, including cancer, frailty, Parkinson's disease, and mortality (41).

Other biological markers of environment-induced age acceleration and new directions

An intensively growing area of current research is epitranscriptomics. While epigenetics corresponds to the study of chemical modifications to DNA, epitranscriptomics focuses on chemical changes to RNA (43). One of the most studied epitranscriptomic marks is N6-methyladenosine (m^6A), which is critical for gene regulation and is modulated by cellular stressors. This mark seems to be the most common epitranscriptomic modification on mRNA, with a presence in 0.1 to 0.4% of all adenines and 20-25%

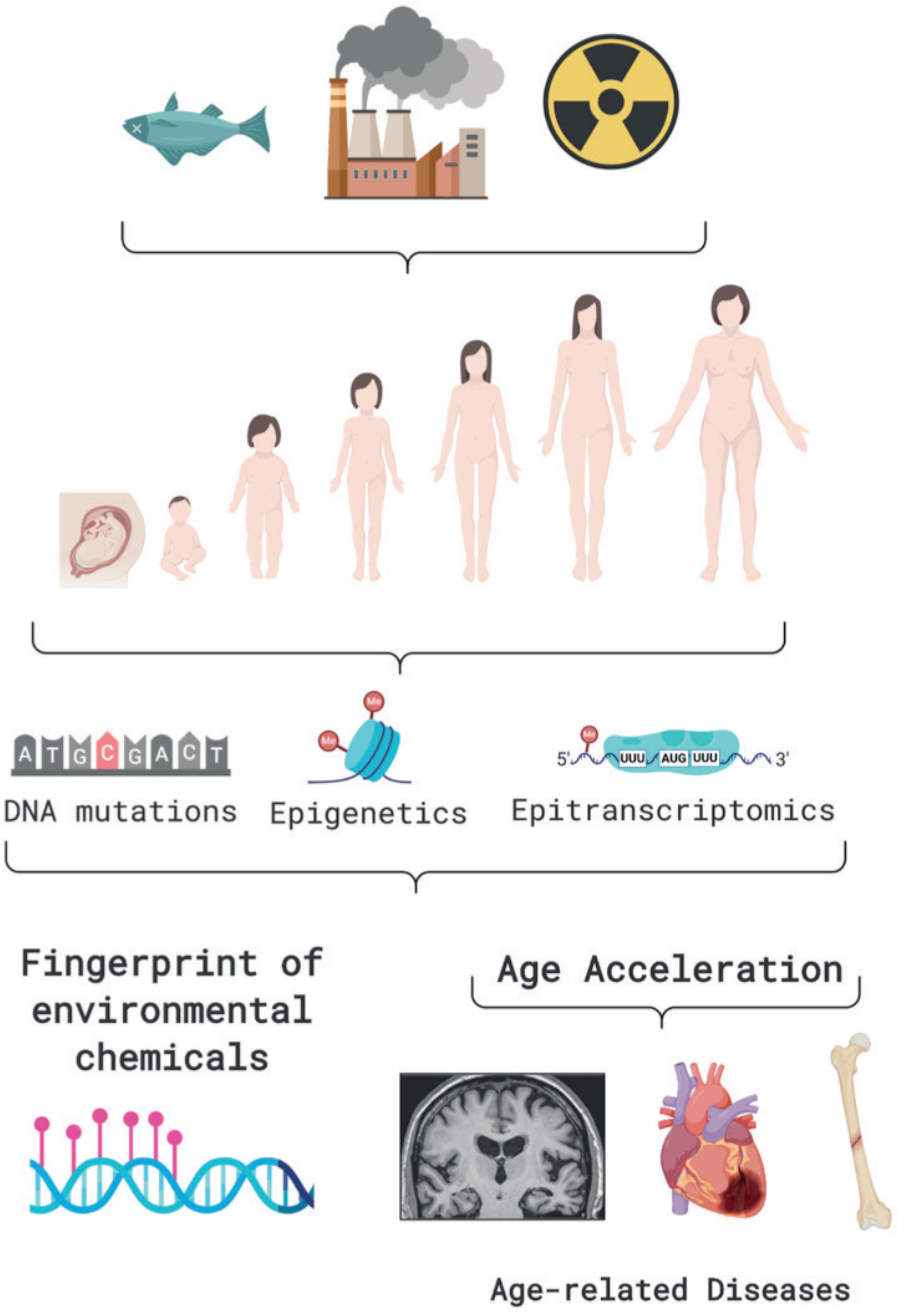

Figure 1: Environmental exposures affect individuals during all life periods and are linked to changes in DNA sequence, epigenetics, and epitranscriptomics, creating exposure fingerprints. These lead to age acceleration and to an increased risk of age-related diseases.

of all transcripts. (44). In a recently published study, our team found that smoking may impact m^6A levels. We found that smoking was associated with a 10.7% reduction in m^6A in peripheral blood, compared with non-smokers (45). Black carbon, a marker of traffic-related air pollution, was associated with higher m^6A levels. Therefore, environmental exposures might generate several molecular changes that can be objectively measured in tissues DNA sequence, regulation, and expression, specifically affecting aging-related processes. Collectively, these molecular signs may help to detect risk of ARDs in time for preventive intervention.

Somatic mutations may also occur in response to environmental-related damage (46). These mutations accumulate in somatic cells during aging, and are referred to as a 'mutation burden' (47). Recent genomic analyses have revealed that the mutation burden in somatic tissues is directly linked with aging (48). Other studies have suggested that environmental exposures are major drivers of mutation load in somatic tissues, particularly in the lungs, suggesting that mutations and age acceleration might be associated with external exposures (49, 50). In fact, extensive evidence is available on the role of air pollutant-derived carcinogens (e.g., polycyclic aromatic hydrocarbons) in the generation of genetic mutations (51). Thus, the mutation burden in somatic cells could also be a strong predictor of biological aging and environmentally-induced cumulative damage in somatic tissues.

Conclusions

Environmental exposures modify our molecules in different ways that have the potential to be used as biomarkers of effect and early predictors of ARDs, including mutations, changes to DNA methylation, the epitranscriptome, and other molecules. These complex relationships between common environmental exposures, molecular changes, and age-related outcomes are shown in **Figure 1**. Current evidence suggests that at least some of these molecular changes can serve as novel biomarkers of cumulative, lifetime exogenous exposure, help capture environmentally-induced age-acceleration, and predict ARDs many years before their diagnosis. Blood-based DNA methylation fingerprinting of exogenous exposures can help identify priority areas for environmental interventions for individual persons. Precise information of age acceleration using molecular biomarkers could aid public health and clinical efforts to reduce effects of toxicants, particularly on ARDs. Additionally, the effect of potential age decelerators (e.g., physical activity, healthy diet, folates, etc.) could also be determined using environmentally-related molecular biomarkers. Molecular measures of accelerated aging may also provide surrogate endpoints to test effectiveness of interventions that aim to extend healthy lifespan by slowing biological processes of aging (52). Such measures could aid in efforts to mitigate health damage from environmental toxicants, serving as surrogate endpoints in studies evaluating impacts of programs and policies to mitigate environmental toxicant burden. More studies incorporating individuals from all life stages, in utero to old age, are needed to study molecular biomarkers and their potential interaction with aging and ARDs.

Acknowledgements

This work was supported by National Institutes of Health [grants R01ES025225, R01ES027747, P30ES009089, and R21ES024236 Baccarelli; R21ES027087 Prada & Baccarelli] and the Consejo Nacional de Ciencia y Tecnologia, CONACYT – Mexico [FOSISS 2017-289503, Prada].

References

1. Grimm SA, Shimbo T, Takaku M, et al. DNA methylation in mice is influenced by genetics as well as sex and life experience. *Nat Commun*. 2019;10(1):305. Published 2019 Jan 18. doi:10.1038/s41467-018-08067-z
2. Belsky DW, Caspi A, Arseneault L, et al. Quantification of the pace of biological aging in humans through a blood test, the DunedinPoAm DNA methylation algorithm. *Elife*. 2020;9:e54870. Published 2020 May 5. doi:10.7554/eLife.54870
3. Franceschi C, Garagnani P, Morsiani C, et al. The Continuum of Aging and Age-Related Diseases: Common Mechanisms but Different Rates. *Front Med (Lausanne)*. 2018;5:61. Published 2018 Mar 12. doi:10.3389/fmed.2018.00061
4. Cerella C, Grandjenette C, Dicato M, Diederich M. Roles of Apoptosis and Cellular Senescence in Cancer

and Aging. *Curr Drug Targets*. 2016;17(4):405-415. doi:10.2174/1389450116666150202155915
5. Cevenini E, Monti D, Franceschi C. Inflamm-ageing. Curr Opin Clin Nutr Metab Care. 2013;16(1):14-20. doi:10.1097/MCO.0b013e32835ada13
6. Jones MJ, Goodman SJ, Kobor MS. DNA methylation and healthy human aging. Aging Cell. 2015;14(6):924-932. doi:10.1111/acel.12349
7. de Haan G, Lazare SS. Aging of hematopoietic stem cells. Blood. 2018;131(5):479-487. doi:10.1182/blood-2017-06-746412
8. Vijg J, Suh Y. Genome instability and aging. *Annu Rev Physiol*. 2013;75:645-668. doi:10.1146/annurev-physiol-030212-183715
9. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. 2013;153(6):1194-1217. doi:10.1016/j.cell.2013.05.039
10. Mc Auley MT, Guimera AM, Hodgson D, et al. Modelling the molecular mechanisms of aging. *Biosci Rep*. 2017;37(1):BSR20160177. Published 2017 Feb 23. doi:10.1042/BSR20160177
11. Rappaport SM, Smith MT. Epidemiology. Environment and disease risks. *Science*. 2010;330(6003):460-461. doi:10.1126/science.1192603
12. Prada D, López G, Solleiro-Villavicencio H, Garcia-Cuellar C, Baccarelli AA. Molecular and cellular mechanisms linking air pollution and bone damage. *Environ Res*. 2020;185:109465. doi:10.1016/j.envres.2020.109465
13. Prada D, Zhong J, Colicino E, et al. Association of air particulate pollution with bone loss over time and bone fracture risk: analysis of data from two independent studies. *Lancet Planet Health*. 2017;1(8):e337-e347. doi:10.1016/S2542-5196(17)30136-5
14. Kulick ER, Wellenius GA, Boehme AK, et al. Long-term exposure to air pollution and trajectories of cognitive decline among older adults. *Neurology*. 2020;94(17):e1782-e1792. doi:10.1212/WNL.0000000000009314
15. Lipfert FW, Wyzga RE. Longitudinal relationships between lung cancer mortality rates, smoking, and ambient air quality: a comprehensive review and analysis. *Crit Rev Toxicol*. 2019;49(9):790-818. doi:10.1080/10408444.2019.1700210
16. Hutchison K, Smith S, Faruqui S. The use of MODIS data and aerosol products for air quality prediction. *Atmospheric Environment*. 2004;38: 5057-5070. DOI: 10.1016/j.atmosenv.2004.06.032
17. Baccarelli AA, Hales N, Burnett RT, et al. Particulate Air Pollution, Exceptional Aging, and Rates of Centenarians: A Nationwide Analysis of the United States, 1980-2010. *Environ Health Perspect.* 2016;124(11):1744-1750. doi:10.1289/EHP197
18. Prada D, González R, Sánchez L, Castro C, Fabián E, Herrera LA. Satellite 2 demethylation induced by 5-azacytidine is associated with missegregation of chromosomes 1 and 16 in human somatic cells. *Mutat Res*. 2012;729(1-2):100-105. doi:10.1016/j.mrfmmm.2011.10.007
19. Zhong J, Karlsson O, Wang G, et al. B vitamins attenuate the epigenetic effects of ambient fine particles in a pilot human intervention trial [published correction appears in Proc Natl Acad Sci U S A. 2017 Apr 18;114(16):E3367]. *Proc Natl Acad Sci U S A*. 2017;114(13):3503-3508. doi:10.1073/pnas.1618545114
20. Peng C, Bind MC, Colicino E, et al. Particulate Air Pollution and Fasting Blood Glucose in Nondiabetic Individuals: Associations and Epigenetic Mediation in the Normative Aging Study, 2000-2011. *Environ Health Perspect*. 2016;124(11):1715-1721. doi:10.1289/EHP183
21. Carmona JJ, Sofer T, Hutchinson J, et al. Short-term airborne particulate matter exposure alters the epigenetic landscape of human genes associated with the mitogen-activated protein kinase network: a cross-sectional study. *Environ Health*. 2014;13:94. Published 2014 Nov 13. doi:10.1186/1476-069X-13-94
22. Rusiecki JA, Beane Freeman LE, Bonner MR, et al. High pesticide exposure events and DNA methylation among pesticide applicators in the agricultural health study. *Environ Mol Mutagen*. 2017;58(1):19-29. doi:10.1002/em.22067
23. Colicino E, Just A, Kioumourtzoglou MA, et al. Blood DNA methylation biomarkers of cumulative lead exposure in adults [published online ahead of print, 2019 Oct 21]. *J Expo Sci* Environ Epidemiol. 2019;10.1038/s41370-019-0183-9. doi:10.1038/s41370-019-0183-9
24. Nwanaji-Enwerem JC, Colicino E. DNA Methylation-Based Biomarkers of Environmental Exposures for Human Population Studies. *Curr Environ Health Rep*. 2020;7(2):121-128. doi:10.1007/s40572-020-00269-2
25. Kennedy BK, Berger SL, Brunet A, et al. Geroscience: linking aging to chronic disease. Cell. 2014;159(4):709-713. doi:10.1016/j.cell.2014.10.039
26. Blackburn EH, Greider CW, Szostak JW. Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. *Nat Med*. 2006;12(10):1133-1138. doi:10.1038/nm1006-1133
27. Holly AC, Melzer D, Pilling LC, et al. Towards a gene expression biomarker set for human biological age. *Aging Cell*. 2013;12(2):324-326. doi:10.1111/acel.12044
28. Peters MJ, Joehanes R, Pilling LC, et al. The transcriptional landscape of age in human peripheral blood. *Nat Commun*. 2015;6:8570. Published 2015 Oct 22. doi:10.1038/ncomms9570
29. Krištić J, Vučković F, Menni C, et al. Glycans are a novel biomarker of chronological and biological ages. *J Gerontol A Biol Sci Med Sci*. 2014;69(7):779-789. doi:10.1093/gerona/glt190
30. Belsky DW, Caspi A, Houts R, et al. Quantification of biological aging in young adults. *Proc Natl Acad*

Sci U S A. 2015;112(30):E4104-E4110. doi:10.1073/pnas.1506264112
31. Jylhävä J, Pedersen NL, Hägg S. Biological Age Predictors. *EBioMedicine*. 2017;21:29-36. doi:10.1016/j.ebiom.2017.03.046
32. Jazwinski SM, Kim S. Examination of the Dimensions of Biological Age. *Front Genet*. 2019;10:263. Published 2019 Mar 26. doi:10.3389/fgene.2019.00263
33. Hannum G, Guinney J, Zhao L, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell*. 2013;49(2):359-367. doi:10.1016/j.molcel.2012.10.016
34. Horvath S. DNA methylation age of human tissues and cell types [published correction appears in Genome Biol. 2015;16:96]. *Genome Biol*. 2013;14(10):R115. doi:10.1186/gb-2013-14-10-r115
35. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet*. 2018;19(6):371-384. doi:10.1038/s41576-018-0004-3
36. Gao X, Colicino E, Shen J, et al. Accelerated DNA methylation age and the use of antihypertensive medication among older adults [published correction appears in Aging (Albany NY). 2019 May 15;11(9):2911]. *Aging (Albany NY)*. 2018;10(11):3210-3228. doi:10.18632/aging.101626
37. Ward-Caviness CK, Nwanaji-Enwerem JC, Wolf K, et al. Long-term exposure to air pollution is associated with biological aging. *Oncotarget*. 2016;7(46):74510-74525. doi:10.18632/oncotarget.12903
38. Hou L, Wang S, Dou C, et al. Air pollution exposure and telomere length in highly exposed subjects in Beijing, China: a repeated-measure study. *Environ Int*. 2012;48:71-77. doi:10.1016/j.envint.2012.06.020
39. Marioni RE, Shah S, McRae AF, et al. DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol*. 2015;16(1):25. Published 2015 Jan 30. doi:10.1186/s13059-015-0584-6
40. White AJ, Kresovich JK, Keller JP, Xu Z, Kaufman JD, Weinberg CR, Taylor JA, Sandler DP. Air pollution, particulate matter composition and methylation-based biologic age. Environ Int. 2019 Nov;132:105071. doi: 10.1016/j.envint.2019.105071. Epub 2019 Aug 3. PMID: 31387022; PMCID: PMC6754788.
41. Nwanaji-Enwerem JC, Weisskopf MG, Baccarelli AA. Multi-tissue DNA methylation age: Molecular relationships and perspectives for advancing biomarker utility. *Ageing Res Rev*. 2018;45:15-23. doi:10.1016/j.arr.2018.04.005
42. Agha G, Mendelson MM, Ward-Caviness CK, et al. Blood Leukocyte DNA Methylation Predicts Risk of Future Myocardial Infarction and Coronary Heart Disease. *Circulation*. 2019;140(8):645-657. doi:10.1161/CIRCULATIONAHA.118.039357
43. Barbieri I, Kouzarides T. Role of RNA modifications in cancer. *Nat Rev Cancer*. 2020;20(6):303-322. doi:10.1038/s41568-020-0253-2
44. Casella G, Tsitsipatis D, Abdelmohsen K, Gorospe M. mRNA methylation in cell senescence. *Wiley Interdiscip Rev RNA*. 2019;10(6):e1547. doi:10.1002/wrna.1547
45. Kupsco A, Gonzalez G, Baker BH, et al. Associations of smoking and air pollution with peripheral blood RNA N6-methyladenosine in the Beijing truck driver air pollution study. *Environ Int*. 2020;144:106021. doi:10.1016/j.envint.2020.106021
46. Lim SM, Choi JW, Hong MH, et al. Indoor radon exposure increases tumor mutation burden in never-smoker patients with lung adenocarcinoma. *Lung Cancer*. 2019;131:139-146. doi:10.1016/j.lungcan.2019.04.002
47. Risques RA, Kennedy SR. Aging and the rise of somatic cancer-associated mutations in normal tissues. *PLoS Genet*. 2018;14(1):e1007108. Published 2018 Jan 4. doi:10.1371/journal.pgen.1007108
48. Yizhak K, Aguet F, Kim J, et al. RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues. *Science*. 2019;364(6444):eaaw0726. doi:10.1126/science.aaw0726
49. Alexandrov LB, Ju YS, Haase K, et al. Mutational signatures associated with tobacco smoking in human cancer. *Science*. 2016;354(6312):618-622. doi:10.1126/science.aag0299
50. Somers CM, Yauk CL, White PA, Parfett CL, Quinn JS. Air pollution induces heritable DNA mutations. *Proc Natl Acad Sci U S A*. 2002;99(25):15904-15907. doi:10.1073/pnas.252499499
51. Agudelo-Castañeda DM, Teixeira EC, Schneider IL, Lara SR, Silva LFO. Exposure to polycyclic aromatic hydrocarbons in atmospheric PM1.0 of urban environments: Carcinogenic and mutagenic respiratory health risk by age groups. *Environ Pollut*. 2017;224:158-170. doi:10.1016/j.envpol.2017.01.075
52. Justice JN, Ferrucci L, Newman AB, et al. A framework for selection of blood-based biomarkers for geroscience-guided clinical trials: report from the TAME Biomarkers Workgroup. *Geroscience*. 2018;40(5-6):419-436. doi:10.1007/s11357-018-0042-y

No potential conflict of interest relevant to this article was reported by the authors