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Epigenetic aging acceleration among World Trade Center-exposed community members

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Aging is a complex biological process, and some individuals are aging faster or slower than expected. This phenomenon of aging acceleration occurs when biological age exceeds chronological age and can be assessed by epigenetic clock estimation. As aging acceleration is known to occur in response to some environmental exposures as well as trauma, we hypothesized that World Trade Center (WTC) exposures may have led to epigenetic aging acceleration. WTC-exposed women were selected from the World Trade Center Environmental Health Center (WTC EHC) clinic, with peripheral blood collected during routine clinical monitoring visits. The reference group was selected from the NYU Women's Health Study (NYUWHS), a prospective cohort study that collected blood samples before 9/11/2001. Epigenomes of WTC-exposed vs. unexposed women were profiled using the Infinium MethylationEPIC array. DNA-based epigenetic aging was estimated using Hannum, Horvath, PhenoAge and GrimAge epigenetic clocks. Age acceleration was defined as the residual from regressing estimated epigenetic age on chronological age. Ordinary least squares regression was used to investigate the relationship between WTC exposure and accelerated aging. After adjustment for race/ethnicity, smoking status, Body Mass Index (BMI), batch and cell type composition, WTC exposure was associated with epigenetic aging acceleration using the Hannum epigenetic clock ($\beta_{\text{WTC Exposed vs. Unexposed}}$: 3.789; p-value: <0.001). WTC exposure was also associated with epigenetic aging acceleration when using other epigenetic clock types (Horvath and PhenoAge, but not GrimAge), and when stratifying by breast cancer case ($\beta_{\text{WTC Exposed vs. Unexposed}}$: 3.473; p-value: <0.001) or cancer-free participant ($\beta_{\text{WTC Exposed vs. Unexposed}}$: 4.369; p-value: 0.001) status. Among all participants, having a breast cancer diagnosis was statistically significantly associated with accelerated aging ($\beta_{\text{Cancer vs. cancer-free}}$: 1.658; p-value: 0.021). WTC exposure is statistically significantly associated with epigenetic aging acceleration. This was true even after stratifying on cancer status. WTC exposure was positively associated with epigenetic aging acceleration in the overall cohort, among only those women who were cancer-free, and among breast cancer cases.

Keywords World trade center, Epigenetics, DNA methylation, Breast cancer

Aging is characterized by the progressive decline in genomic and system integrity and is itself a major driver of many chronic diseases[1]. There are multiple mechanisms of aging, and hallmarks of aging can be characterized based on damage to the genome, the physiological response to this damage, and the results from accumulation of damage beyond a point that the body can compensate for; this complexity and nuance in the aging process is indicated by the heterogeneity in health among persons of the same chronological age[2]. Some individuals may actually be aging faster than expected. This phenomenon of aging acceleration occurs when biological age exceeds chronological age. Persons exposed to the World Trade Center (WTC) disaster, including rescue and recovery workers (responders) and community members (survivors) may be experiencing aging acceleration given that WTC exposure is associated with greater age-related vulnerability. This is evidenced

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by the excess burden of aging-related conditions in this group, including frailty. Almost one-third of the WTC responders meets the criteria for frailty under a cumulative deficit model, a syndrome of increased age-related vulnerability that manifests as mortality, morbidity, disability, hospitalizations, and unfavorable outcomes after surgical procedures[3]. Frailty can also be conceptualized as the diminished ability to maintain normal function/homeostasis[4]. Age-related morbidity is also increased among WTC responders. Specifically, cancer in general, and rates of certain cancers, including prostate, thyroid, melanoma and tonsil cancer, are elevated among responders[5–9], and cancer, including breast cancer, is commonly observed among WTC survivors[10,11]. WTC-associated cancers may also be, on average, more aggressive than cancers of WTC-unexposed persons[12,13]. Responders and survivors also suffer an excess burden of other aging-related syndromes and morbidities such as compromised lung function and increased asthma incidence, decreased sleep quality, mental health, and cognitive functioning impairment, and increased risk of autoimmune diseases, among other adverse health impacts[14,15]. Increased WTC exposure is additionally associated with increased risk of mortality[16]. Moreover, WTC responders and survivors experienced intense psychological trauma. Risk of post-traumatic stress disorder (PTSD) is elevated among WTC responders[17], and PTSD and trauma are associated with immune system dysregulation[18] and premature aging[19].

Despite mounting epidemiological evidence of the WTC-associated premature aging, studies are needed that explore this on the biological level. Epigenetic clocks are commonly used to estimate biological aging through DNA methylation-derived changes. DNA methylation occurs when there is an addition of a methyl group at a cytosine-phosphate-guanine (CpG) site[20,21]. When DNA methylation occurs at gene promotors, this will often result in silencing of gene expression[21]. Dynamic and actively maintained throughout the genome, DNA methylation is sensitive to environmental stimuli, acting as an interface between the environment and the genome[21]. Epigenetic clocks use DNA methylation levels at CpG sites throughout the genome to estimate biological age, and are generally useful predictors of biological aging across the lifespan[22], with some epigenetic clocks, like GrimAge, specifically trained on mortality data[23]. Epigenetic aging acceleration can occur in response to environmental exposures[1]. Exposure to endocrine-disrupting chemicals[1], air pollution[24–26], metals[26], and dioxins[27], found in the WTC dust, have all been linked to aging acceleration. WTC dust comprised damaging toxicants including metals (Arsenic [As], Beryllium [Be], Cadmium [Cd], Chromium [Cr] and Nickel [Ni]), asbestos, polycyclic aromatic hydrocarbons (PAHs), persistent organic pollutants (POPs, including polychlorinated biphenyls [PCBs] and dioxins), volatile organic compounds (VOCs, including benzene), and endocrine disrupters like per- and polyfluoroalkyl substances (PFAS)[5,9,28]. These environmental exposures are thought toimpact aging by modifying molecular mechanisms, such as mitochondrial metabolism and inflammation[1].

Aging acceleration has important health implications. It is inversely correlated with physical and cognitive fitness[29–32], and accelerated aging increases risk of mortality, independent of chronological age[33]. For each 1-year increase in aging acceleration, there is a 6% risk of developing cancer within 3 years and a 17% increase in the risk of dying from cancer within 5 years[34].

To summarize, we hypothesize that WTC exposure may be associated with premature aging given: (1) aging-related vulnerability and mortality is increased among WTC-exposed persons; (2) trauma and PTSD, which are associated with premature aging, are also common among WTC-exposed individuals; and (3) many of the toxicants found in the WTC dust are known to be associated with epigenetic dysregulation. The main objective of the current study was to investigate epigenetic aging acceleration of WTC-exposed persons using previously validated epigenetic clocks.

Materials and methods

Study participants and samples

WTC-exposed women were selected from the World Trade Center Environmental Health Center (WTC EHC). The WTC EHC acts as the "Center of Excellence" for the treatment and surveillance of WTC-affected local community members, also known as survivors[10,35]. WTC survivors include "persons who were present in the dust or dust cloud on 9/11 or who worked, lived, or attended school, childcare centers, or adult day care centers in the NYC disaster area"[35]. Patients self-enroll into the WTC EHC program and are required by law to have a "certifiable condition", such as a respiratory condition or cancer, or some other condition, in addition to WTC exposure[15,35,36]. Certifiable conditions are those determined by the WTC Health Program as WTC-related; more information can be found at https://www.cdc.gov/wtc/conditions.html. Only women who were cancer-free or who had breast cancer were included in this study. More than 6000 cancer patients, including 1300 breast cancer patients, have been diagnosed as of November 1, 2023[10,11]. Physical examinations, mental health screens, blood tests, and chest X-rays are routinely performed at the WTC EHC Clinic. Participants were consented and blood was collected during routine clinical visits. WTC EHC subjects' blood samples were collected in 2022–2023[37,38]. Relevant clinical and sociodemographic data were collected from both initial and monitoring visits using interviewer-administered questionnaires[10,11].

WTC-unexposed controls were selected from the NYU Women's Health Study (NYUWHS). The NYUWHS is a prospective cohort in which women between the ages of 35 and 65 years old were enrolled between March 1985 and June 1991 at the Guttman Breast Diagnostic Institute in New York City[39]. Subjects completed a self-administered baseline questionnaire on demographic, medical, reproductive, regular physical activity, and recent medication use. All cohort participants donated peripheral blood, which was subsequently placed in long-term storage at − 80 °C. Because the sample collection predated 9/11/2001, all preserved NYUWHS blood samples are guaranteed to be WTC-unexposed. Participants have been followed up regularly since enrollment, to identify incident cancer cases. To match NYUWHS inclusion criteria, only those WTC EHC participants who were women between the ages of 35–65 years old, who were not pregnant or breastfeeding, and who had not been pregnant or breastfeeding in the 6 months preceding study enrollment, were included in this analysis. WTC-exposed and unexposed women were frequency matched on age. This study relied on convenience sampling of

WTC-exposed women with breast cancer. Women being seen at the WTC EHC clinic for their annual monitoring visit were approached for study participation by study personnel (ST) provided they met these inclusion criteria. Study eligibility was determined by the clinical team (lead by JR). Women were consented and enrolled in the clinic until the target number of 96 was reached. Women who agreed to participate had an additional vial of blood collected during their routine bloodwork. This study had a 192-target final sample size (96 WTC-exposed and 96 unexposed participants).

Epigenome-wide profiling and epigenetic clock estimation

We used established methods for DNA processing and DNA methylation profiling as described more fully elsewhere[37,38]. Briefly, blood samples were sent to the NYU Langone Health Center for Biospecimen Research and Development (CBRD) laboratory for DNA extraction from white blood cells and treated with bisulfite to convert unmethylated cytosines to uracil, allowing for the identification of DNA methylation patterns at single-base resolution. Following the manufacturer's protocol, the Illumina Infinium MethylationEPIC BeadChip version 2.0 (EPICv2) (Illumina®) was used to determine the DNA methylation status of 866,562 CpG sites. Participants samples were run using two EPIC BeadChips kits, each balanced for WTC-exposed and unexposed samples, randomized across array chips and scanned within the same time frame. We used the "ComBat" function from the "sva" R package to perform batch correction using the Sentrix slide ID as the batch variable. Each slide corresponds to a unique Illumina BeadChip processing 8 samples, and this correction effectively addresses both between-kit and within-kit (slide-level) technical variation. The R package "minfi" was used to process and analyze methylation data. Probes were quantile normalized and the background adjusted, using functions "preprocessQuantile" and "preprocessNoob[40,41]. Normalized DNA methylation data at various CpG sites was used to calculate epigenetic clocks, estimated using the DNA Methylation Age calculator (available at: https://dnamage.clockfoundation.org/). This included the Horvath[42], Hannum[43], PhenoAge[32] and GrimAge[23,44] epigenetic clocks. GrimAge version 2 was used. These epigenetic clock models are capturing different DNA methylation age-predictive factors. For instance, the Horvath and Hannum clocks have only 6 CpG sites in common[22], and so utilizing various methodologies ensures a more robust investigation of the study hypothesis. Differences between these epigenetic clocks are described in Supplementary Table S1. In terms of presented results, the Hannum clock epigenetic age was prioritized as it is the original blood-based method of epigenetic age estimation and has been the widely validated and used type[45], and because it is correlated with chronological age. In contrast, while Horvath is likewise correlated with chronological age, it is tissue-based, and PhenoAge and GrimAge correlate with aging-related morbidity and mortality, respectively (Supplementary Table S1). Later generation epigenetic clocks were not considered here but should be utilized in future.

Notably, the ability of epigenetic clocks to reflect biological aging is chronological age dependent. Specifically, epigenetic clocks are less effective at predicting biological age among older individuals, and may actually underestimate biological age due to the phenomenon of saturation, i.e., when CpG sites reach either full methylation or complete demethylation[46,47]. Defining age acceleration as the residual from regressing estimated biological age on chronological age (residuals (lm (epigenetic age - chronological age))) is one method to correct for this issue[47]. The full model is illustrated as follows: $Y = \beta 0 + \Sigma j = 1.p\ \beta jXj + \varepsilon$, where Y is the residual of epigenetic age on chronological age, β0 is the value of Y when X (WTC exposure) is equal to zero (unexposed), and βj is the slope, or change in Y when X (WTC exposure) is equal to 1 (exposed), adjusted for confounding factors. Epigenetic accelerated aging when calculated as the residual variation in epigenetic age independent of chronological age is "a measure of how much an individual is aging faster or slower than their chronological age"[46].

Statistical analysis

Chronological and DNA methylation-derived age were summarized as continuous variables. T-tests were used to compare means for chronological and epigenetic ages between WTC-exposed and unexposed groups. Factors associated with WTC exposure status, including race, smoking status, BMI, were assessed using the Pearson test for normal and categorical variables and the Spearman test for continuous non-normal variables. These variables were chosen as each is known to influence DNA methylation[48–50]. Methylation-derived age acceleration is defined as positive residual variation in biological age independent of chronological age. Unadjusted and adjusted Ordinary Least Squares (OLS) regression was used to assess differences in accelerated aging according to the WTC exposure status (WTC-exposed vs. unexposed). In the adjusted model the following covariates were included: breast cancer status (post-diagnostic breast cancer case [WTC EHC] or pre-diagnosis case [NYUWHS] vs. control), race/ethnicity (Asian, Hispanic, Non-Hispanic Black, Non-Hispanic White, Other, Unknown), BMI (healthy [defined as BMI 18.5 to < 25], underweight [defined as BMI < 18.5], overweight [defined as BMI 25.0 to < 30], obese [defined as BMI ≥ 30, unknown), smoking status (ever, never), and education status (≤ high school/vocational school, attended college or graduate school, unknown). We used the estimateCellCounts() function in the minfi package to estimate the proportions of major immune cell types (CD8 + T, CD4 + T, NK, B cells, monocytes, and granulocytes). These estimates were then included as covariates in our regression models[51]. This approach—incorporating cell-type proportions as model covariates rather than adjusting methylation values directly—is a widely used and recommended strategy. Given the proportional nature of the cell type counts, granulocytes were not incorporated into the models. Data was also batch-adjusted ($n_{batches} = 2$) based on which EPIC BeadChip kit was used. Participants with missing data for covariates were not included in the adjusted models. All statistical analyses were done using *R* statistical software[52].

Results

Initially samples were acquired for 192 participants, enough for two Illumnia BeadChips. DNA was successfully extracted, and epigenetic clocks were estimated for 189 participants, 4 of which had to be excluded as

	WTC-exposed ($n=105$)	WTC-unexposed ($n=80$)	p-value
Age at sample donation in years, mean (standard deviation)	58.4 (7.6)	53.3 (7.2)	< 0.001
Race/ethnicity, n (%)			0.008
Hispanic	16 (15.2%)	7 (8.8%)	
Non-Hispanic black	27 (25.7%)	12 (15.0%)	
Non-Hispanic white	47 (44.8%)	56 (70.0%)	
Other/unknown	15 (14.3%)	5(56.3%)	
Smoking status, n (%)			0.022
Ever	32 (30.5%)	38 (47.5%)	
Never	70 (66.7%)	39 (48.8%)	
BMI category, n (%)			0.064
Normal weight (< 25)	24 (22.9%)	35 (43.8%)	
Overweight (25 < = BMI < 30)	26 (24.8%)	27 (33.8%)	
Obese (BMI > = 30)	31 (29.5%)	18 (22.5%)	
Cancer status			0.492
Cancer-free	46 (43.8%)	40 (50.0%)	
Breast cancer	59 (56.2%)	40 (50.0%)	
Batch			0.031
Batch 1	45 (42.9%)	48 (60.0%)	
Batch 2	60 (57.1%)	32 (40.0%)	

Table 1. Descriptive characteristics of the WTC-exposed vs. unexposed participants. *Smoking status missing for $n=6$ (3 WTC-exposed and 3 unexposed) participants; BMI missing for $n=24$ WTC-exposed participants.

	WTC-exposed	WTC-unexposed	p-value
Chronological age	58.4 (7.6)	53.3 (7.2)	< 0.0001
Hannum age	68.9 (6.3)	63.0 (6.6)	< 0.0001
Horvath age	65.3 (5.8)	61.1 (7.0)	< 0.0001
PhenoAge	52.0 (8.0)	48.0 (8.4)	0.0015
GrimAge	74.9 (6.5)	74.0 (6.3)	0.3402

Table 2. Age according to epigenetic clocks (in years; means and standard deviations). *β coefficients for WTC exposed vs. unexposed from the statistical OLS models; denotes statistical significance ($p<0.05$); Adjusted for race, BMI, smoking status, cancer status, cell type composition (CD8T cells, CD4T cells B cells, NK cells, B cells, Monocytes), batch. Unit is years.

chronological age was unknown; of those remaining, 105 were WTC-exposed and 80 WTC-unexposed[37,38,53]. Characteristics of these participants have been described in detail elsewhere[37,38,53]. Notably, WTC-exposed vs. unexposed women differed meaningfully in race/ethnicity, smoking status, and BMI. Compared to the unexposed women, WTC-exposed participants were more racially and ethnically diverse (44.8% vs. 70.0% non-Hispanic white), were more likely to be never smokers (66.7% vs. 48.8%) and have higher BMIs (obese: 29.5% vs. 22.5%) (Table 1). These factors were included in the adjusted models, in addition to cell type composition, cancer status, and batch.

The mean chronological age at blood donation for the entire cohort was 56.4 years old. Although WTC-exposed and unexposed women were frequency-matched on age, women with WTC exposure were, on average, slightly older (mean chronological age: 58.4, standard deviation [SD]: 7.6 years) than; unexposed women (mean chronological age: 53.3, SD: 7.2 years). For each of the epigenetic clock methods, the mean estimated epigenetic age was higher among WTC-exposed vs. unexposed participants: Hannum: 68.9 vs. 63.0; Horvath: 65.3 vs. 61.1; PhenoAge: 52.0 vs. 48.0; GrimAge: 74.9 vs. 74.0. Univariate analysis showed that between WTC-exposed and unexposed women, mean chronological age, Hannum ($p<0.0001$), Horvath ($p<0.0001$) and PhenoAge ($p=0.0015$) ages were statistically significantly different, but not for GrimAge (Table 2; Fig. 1). The average residual for aging acceleration was Hannum: 1.10 for WTC-exposed and − 1.10 for WTC-unexposed; Horvath: 0.367 for WTC-exposed and − 0.389 for WTC-unexposed; PhenoAge: 0.419 for WTC-exposed and − 0.025 for WTC-unexposed; and GrimAge: − 0.376 for WTC-exposed and 0.517 for WTC-unexposed.

Compared with WTC-unexposed women, women with WTC exposure had accelerated aging using Hannum clock both before ($\beta_{\text{WTC Exposed vs. Unexposed}}$: 2.198; p-value: 0.001) and after ($\beta_{\text{WTC Exposed vs. Unexposed}}$: 3.789; p-value: <0.001) adjustment. In adjusted models, WTC exposure was also associated with accelerated aging using Horvath ($\beta_{\text{WTC Exposed vs. Unexposed}}$: 2.201; p-value: 0.009) and PhenoAge ($\beta_{\text{WTC Exposed vs. Unexposed}}$: 2.185; p-value: 0.045) methods, but not GrimAge epigenetic clocks ($\beta_{\text{WTC Exposed vs. Unexposed}}$: 0.449; p-value: 0.418) (Fig. 2). Among all participants, having a breast cancer diagnosis was statistically significantly associated with

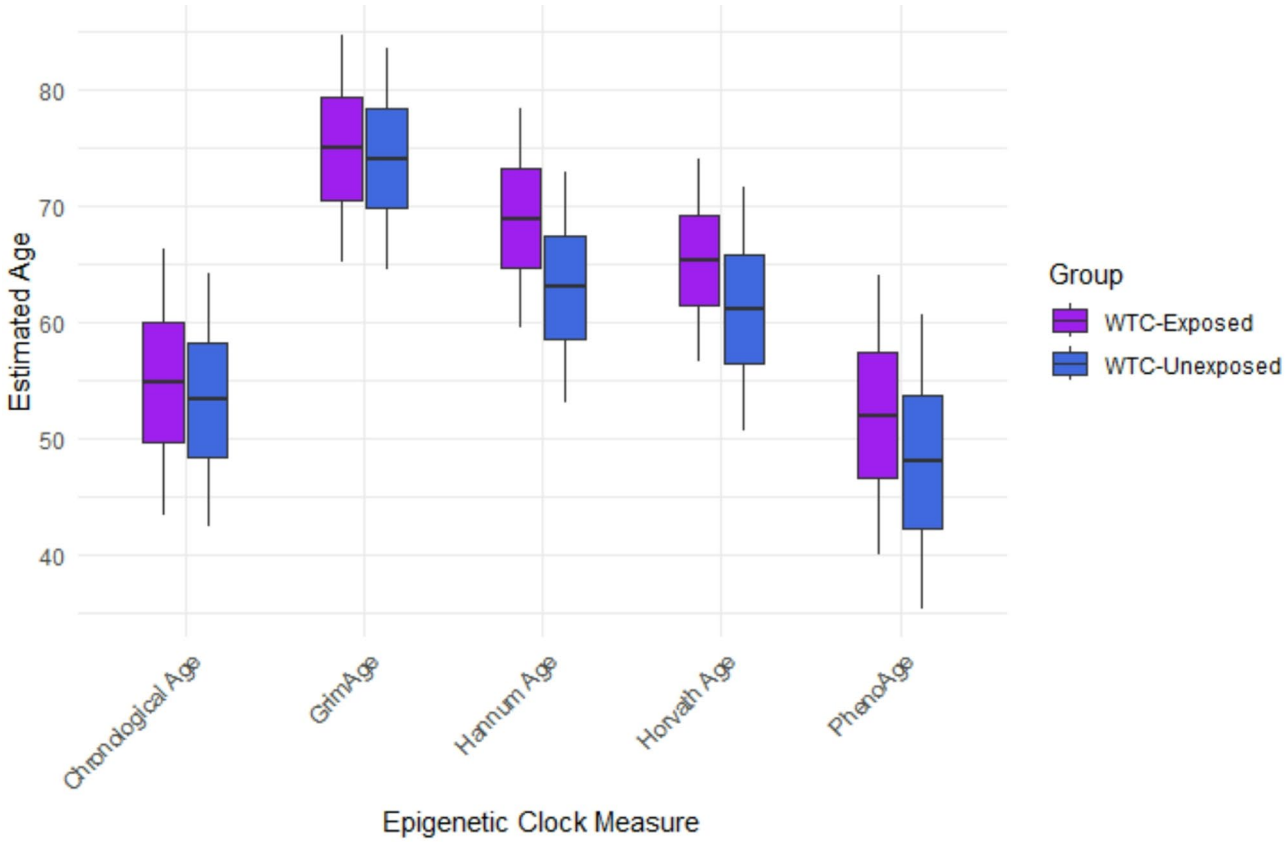

Fig. 1. Epigenetic clock ages by WTC exposure status.

accelerated aging ($\beta_{\text{Cancer vs. cancer-free}}$: 1.658; p-value: 0.021; Hannum epigenetic clock). When stratified, WTC exposure was statistically significantly associated with accelerated aging in both breast cancer cases (adjusted $\beta_{\text{WTC Exposed vs. Unexposed}}$: 3.473; p-value: <0.001) and cancer-free participants (adjusted $\beta_{\text{WTC Exposed vs. Unexposed}}$: 4.369; p-value: 0.001). Full model results for the Hannum epigenetic clock are reported in Supplementary Table S2.

Discussion

Accelerated aging due to complex environmental exposures is an understudied phenomenon. We demonstrated that measuring biological aging using DNA methylation and epigenetic clocks can improve our understanding of age-related vulnerability among WTC-exposed individuals. We also present preliminary evidence that WTC exposure may be associated with accelerated aging. Women with WTC exposure, on average, appear to be aging more rapidly compared to unexposed persons according to several epigenetic clocks.

It is currently uncertain what aspects of aging epigenetic clocks represent. One possibility is that epigenetic clocks reflect an evolutionarily conserved and highly regulated process whereby DNA methylation rates change throughout the life course[31,42]. It's also possible that epigenetic clocks are capturing cumulative lifetime damage to DNA[54]. These theories are not mutually exclusive, and accelerated aging could represent a deviation from normal processes and/or an accumulation of DNA damage. A better understanding of what biological aspects are being captured by different epigenetic clocks, and how they can be dysregulated, may help clarify how environmental exposures may accelerate aging. Notably, the different methods of epigenetic clock estimation showed considerable variability, especially the PhenoAge and GrimAge clocks. Future work is needed to better understand which type of clocks are best for studying the impact of environmental exposures on biological aging.

This work, while preliminary, is consistent with past findings. A large body of work from our and other groups has demonstrated that global and site-specific DNA methylation dysregulation is common among WTC-exposed individuals[37,38,53,55]. While this is the first study to use epigenetic clocks to compare rates of accelerated aging among WTC-exposed vs. unexposed participants, in a recent analysis of WTC responders with and without current PTSD, GrimAge methylation age acceleration was observed to be associated with PTSD diagnosis, although PhenoAge, Hannum, and Horvath methylation age acceleration were not robustly related to PTSD[56]. RNA-seq results corroborate this finding; responders with current PTSD, compared to those without, showed accelerated transcriptional aging, even after adjustment for chronological age[56]. An analysis of post-9/11 United States military veterans found that veterans with current PTSD were aging faster than those without current PTSD, assessed using DNA methylation measures of epigenetic aging[19]. Multiple other studies

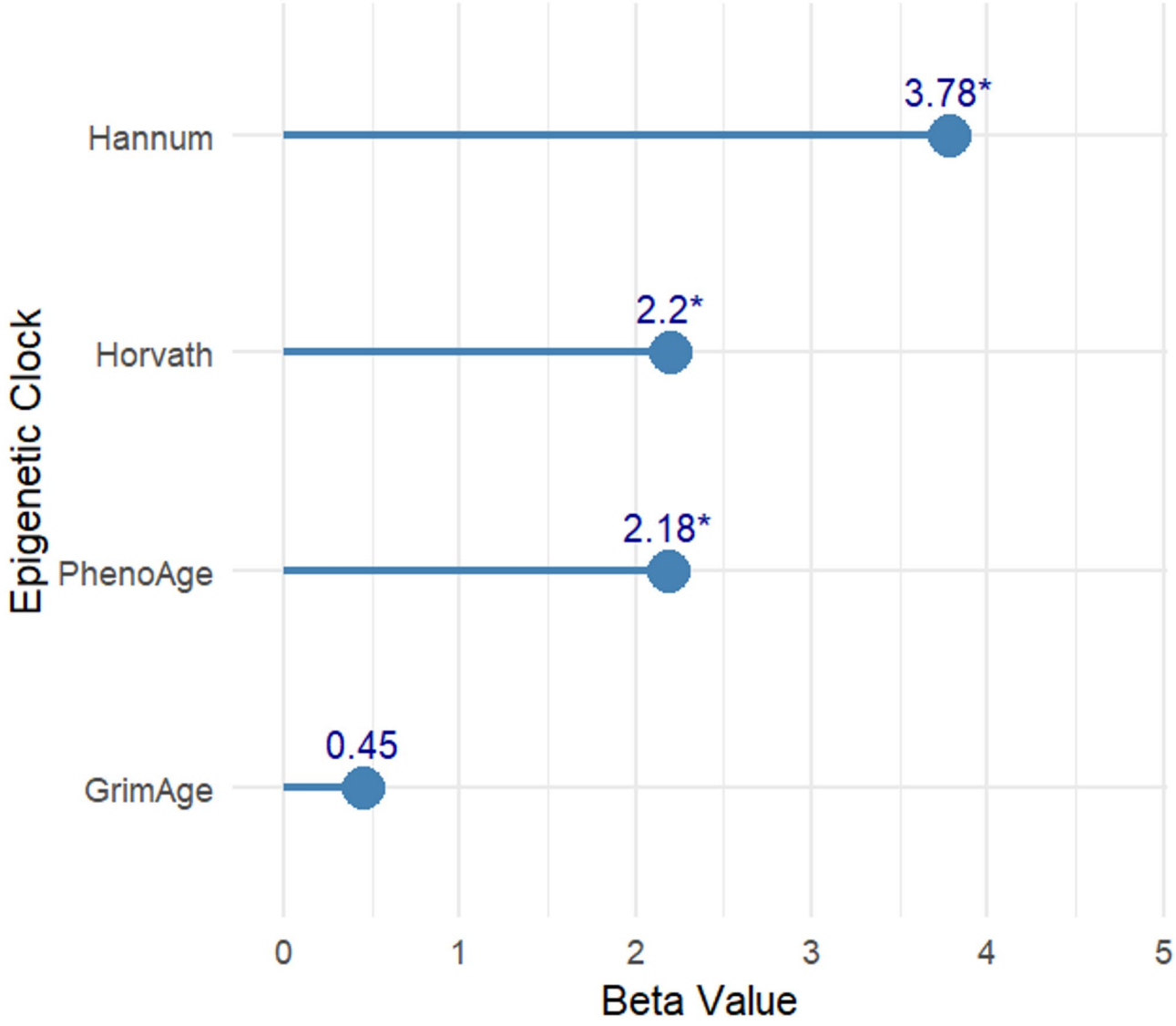

Fig. 2. Accelerated aging of WTC-exposed vs. unexposed participants.

confirm the link between trauma, PTSD, and epigenetic aging acceleration[57–59], possibly due to an increase in inflammation and oxidative stress, that induce cell division and lead to telomere shortening[60]. Biological aging acceleration has therefore previously been documented among WTC responders and similar groups; however, this is the first study to attempt to quantify the degree to which WTC-exposed vs. unexposed groups differ in their aging processes. Future work is needed to expand on these results to validate the findings reported here, and to elucidate the ways in which accelerated aging may be adversely affecting the health of WTC-exposed responders and survivors.

Strengths of our study included the use of a WTC-unexposed control group, the use of multiple epigenetic clock methods, a statistical design that incorporated baseline chronological age into the estimation of biological aging, and adjustment for important confounding factors, such as smoking status and BMI, as well as cell type composition. This is also the first study focused on WTC-exposed women. This is important as it's well established that sex is an effect measure modifier of both DNA methylation profiles and the normal aging process[61].

Several study limitations should be considered. This work was based on relatively small project epigenetically profiling WTC-exposed vs. unexposed women who were either cancer-free or had breast cancer[53]. Women with other cancer types were excluded, and thus we cannot address the impact of WTC exposure on epigenetic aging among other cancer phenotypes. While women with breast cancer had blood donated close to their diagnoses, and so should have been treatment naive, we cannot discount an impact of cancer treatment on epigenetics and therapy-induced aging[62]. Also notable, this study relied on convenience sampling, which has inherent limitations which could impact validity and generalizability. The possibility of selection bias cannot be excluded, and WTC survivors who were more health conscious, and more likely to visit the WTC EHC clinic, may be overrepresented in our study sample. A larger study based on a random sample of WTC survivors is warranted. A statistically significant relationship between WTC exposure and accelerated aging as measured

by GrimAge was not observed. While the fact that the Hannum, Horvath and PhenoAge epigenetic clocks all yielded statistically significant results increases confidence in our study findings, it should be noted that these clocks were designed to capture different aspects of the biological aging process, and which clock, if any, is best for capturing the effect of environmental exposures on aging remains a matter of ongoing research. GrimAge, correlated to mortality data, may be capturing different aspects of the biological aging process that are less impacted by WTC exposure. A larger sample size of WTC-exposed individuals may also allow for an investigation into a dose-response relationship between WTC exposure and accelerated aging, which would further support the study's hypothesis. Additionally, while our results highlight the need to adjust for confounding factors when investigating accelerated aging, unmeasured confounding may persist. Socioeconomic status, specifically, is likely an important consideration. Education status was available for a subset of subjects, which can be used as a proxy for income. In a sensitivity analysis of subjects with known education status, WTC exposure was still associated with accelerated aging, even after adjustment for education (Hannum, adjusted $\beta_{\text{WTC Exposed vs. Unexposed}}$: 6.445; p-value: <0.001; data not shown). Future studies with more detailed clinical and sociodemographic data are nevertheless warranted. WTC-exposed and unexposed samples differed by sample storage time, with WTC-unexposed NYUWHS samples having been collected decades before the analysis. Stability of white blood cell DNA methylation profiles, however, is robust. It has been reported previously that blood samples stored for long periods at − 80 °C have stable DNA methylation profiles[63]. DNA extracted from the blood thawed 20 years after it was frozen at − 80 °C has been shown to have stable DNA methylation levels at different genomic regions (TSS, promoter, gene body, CGI, and CGI shore regions)[64]. It is a notable limitation that we cannot discern the effects of specific components of the WTC toxic dust, and trauma, on epigenetic aging, which should be the focus of future research. Future studies should explore this relationship in WTC-exposed males, including WTC rescue and recovery workers, who may have had higher levels of acute WTC dust exposure. Lastly, germline genetics likely also plays a role in accelerated aging, however, participants genotypes were unknown and sample size was too small to investigate gene-gene and gene-environment effects.

Conclusions

WTC-exposed persons appear to be experiencing accelerated epigenetic aging compared to unexposed individuals. Most WTC responders are now at a greater chronological age and at an increased risk of aging-related chronic diseases. To date, the biological mechanisms underpinning WTC exposure-disease relationships remain understudied, but premature aging may play a role. Improved understanding of how WTC exposure modifies the biological aging processes could improve both preventive and therapeutic approaches for WTC-exposed persons. Biomarkers of accelerated aging can also be used to screen for age-related vulnerability and syndromes. The results presented here, while preliminary, could have important implications not just for WTC-exposed persons but also for individuals exposed to other complex environmental exposures.

Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

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Author contributions

Conceptualization: A.A.A., M.S. and Y.S.; Data curation: S.T.; Formal analysis: S.T., Y.A., C.S.; Funding acquisition: A.A.A., J.R.; Investigation: S.T., Y.A., C.S., S.R; Methodology: S.T., A.A.A., Y.A., Y.S., R.S., C.S., and M.S.; Project administration: S.T., A.A.A.; Resources: A.A.A., M.S., and J.R.; Supervision: A.A.A., M.S. and Y.S.; Visualization: S.T. and Y.A. All authors have made substantial contributions to Writing - original draft and Writing - review & editing. All have final approval of the version to be submitted.

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Declarations

Competing interests

The authors declare no competing interests.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Boards of NYU Grossman School of Medicine and Bellevue Hospital (IRB numbers: s17-01207 and i21-00717). Informed consent was obtained from all study participants.

Additional information

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