Epigenetic Age Acceleration and Chronic Health Conditions Among Adult Survivors of Childhood Cancer

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Abstract

Background: Mounting evidence supports the occurrence of accelerating aging among long-term survivors of childhood cancer. We aimed to investigate epigenetic age acceleration (EAA) in survivors and evaluate associations between EAA, treatment exposures, health behaviors, and chronic health conditions (CHCs). **Methods:** Genome-wide methylation data were generated with Infinium EPIC BeadChip on blood-derived DNA from 2139 survivors and 282 frequency matched controls from the St Jude Lifetime Cohort Study. EAAs were estimated as residuals from a linear regression of epigenetic age (Levine's clock) against chronological age. Adjusted least square mean (ALSM) of EAA was calculated and compared between survivors and controls, across treatment exposures and health behaviors. Associations of EAA with 20 clinically assessed CHCs were evaluated with multivariable piecewise-exponential models. All statistical tests for *P* values below were 2-sided. **Results:** EAA was statistically significantly higher in survivors than controls (ALSM = 0.63, 95% confidence interval [CI] = 0.26 to 1.01 vs −3.61, 95% CI = −4.43 to 2.80). In a multivariable model among survivors, statistically significantly higher EAA (*P* < .05) was observed in those exposed to chest radiotherapy, abdomen or pelvic radiotherapy, alkylating agents, glucocorticoids, or epipodophyllotoxins. Compared with survivors with favorable health behaviors (ALSM = 0.26, 95% CI = −0.36 to 0.87), EAA was statistically significantly higher among survivors with intermediate (ALSM = 1.07, 95% CI = 0.59 to 1.54) or unfavorable health behaviors (ALSM = 1.45, 95% CI = 0.60 to 2.30). In time-to-event analyses, statistically significant associations were identified between EAA tertiles and incidence of 7 CHCs: hypertension (3rd vs 1st tertile, relative rate [RR] = 1.83, 95% CI = 1.17 to 2.83), myocardial infarction (RR = 2.91, 95% CI = 1.27 to 7.21), obesity (RR = 1.39, 95% CI = 1.17 to 1.66), obstructive pulmonary deficit (RR = 1.86, 95% CI = 0.95 to 3.77), peripheral motor neuropathy (RR = 2.89, 95% CI = 1.24 to 6.97), peripheral sensory neuropathy (RR = 2.04, 95% CI = 0.99 to 4.26), and pulmonary diffusion deficits (RR = 2.75, 95% CI = 0.95 to 7.63). **Conclusions:** EAA is statistically significantly higher in survivors of childhood cancer than in noncancer controls and is associated with specific treatment exposures, unfavorable health behaviors, and presence of specific CHCs.

With progress in cancer treatment and increased life expectancy, the population of childhood cancer survivors in the United States has grown and is now estimated to include over 500 000 (1). Unfortunately, therapeutic agents and modalities

used to treat childhood cancers have long-term consequences on healthy tissues and organs (2). Adult survivors of childhood cancer are at risk of treatment-related adverse health outcomes, including a broad range of chronic health conditions (CHCs) (3),

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hospitalizations (4), premature frailty (5), and early mortality (6). Moreover, the prevalence of physiological frailty, an aging phenotype commonly observed in older adults (7), is higher in childhood cancer survivors compared with their similarly aged siblings (8) or comparable between young adult survivors (in their 30s) and much older noncancer individuals (in their 60s) (5). As a suggestive sign of accelerated aging, risk for CHCs is threefold higher among survivors than their similarly aged siblings (9). Our recent study showed survivors had statistically significantly shorter leukocyte telomere length (LTL) than controls, where the mean LTL of 6 Kb corresponded to an age of 36.8 years in survivors compared with 48.2 years among controls, suggesting an approximately 11-year acceleration in telomere attrition among survivors (10,11). Thus, understanding modifiable risk factors associated with premature aging among childhood cancer survivors is a major priority for both prevention and remediation of CHCs.

Aging is a complex and progressive process characterized by various changes at both molecular and cellular levels, such as telomere attrition, mitochondrial dysfunction, and epigenetic alterations (12). Telomere length is generally considered as a promising aging biomarker; however, the high variability across detection methods and weak correlations with age-related outcomes limit its use as an unambiguous biomarker of aging (13,14). Recently, increasing evidence suggests that dynamic DNA methylation, the most studied durable and reversible epigenetic alteration, outperforms other aging biomarkers in its strong correlations with aging and longevity (15). Measures of epigenetic age acceleration (EAA), the discrepancy between estimated epigenetic age and chronological age, can be a good proxy for the general health status or rate of physiological aging (16). To track such age-dependent epigenetic changes, several DNA methylation-based epigenetic clocks have been proposed (16–18), among which the Levine's clock (ie, DNA methylation phenotypic age [DNAmPhenoAge]) provides superior predictive accuracy for time to death, all-cause mortality, and health span in the general population (15). The acceleration of DNAmPhenoAge also shows strong associations with a variety of age-related CHCs across different tissues and organs, including cardiovascular diseases, dementia, and frailty (18).

To date, the potential utility of EAA as a biomarker of aging among survivors of childhood cancer has not been established. To address this knowledge gap, we conducted genome-wide methylation profiling on blood-derived DNA from participants from St Jude Lifetime Cohort Study (19). EAA was compared between survivors and noncancer controls as well as assessed for associations with cancer treatment exposures, modifiable health behaviors, and clinically ascertained CHCs.

Methods

Study Population

A total of 2421 St Jude Lifetime Cohort Study participants of European ancestry were included, consisting of 2139 childhood cancer survivors and 282 community controls with no history of cancer. Controls were frequency-matched to the survivors by age, sex, and race. For comparison of EAA between survivors and controls, the analysis was limited to 1563 survivors and 282 controls whose DNA extraction was completed with the same protocol in the Computational Biology Genomic Laboratory at St Jude Children's Research Hospital (Supplementary Methods, available online).

Chronic Health Conditions

A modification of the Common Terminology Criteria for Adverse Events (version 4.03, National Cancer Institute) (20) was applied to clinically ascertained medical outcomes and score for severity (19). Clinical outcomes were severity graded as 0 (no problem), 1 (mild), 2 (moderate), 3 (severe or disabling), and 4 (life-threatening) (19). Twenty common (incidence ≥ 20) nonneoplastic CHCs graded as 3–4 were considered for statistical analyses in this study.

Treatment Exposures

Treatment exposure information was extracted from medical records using a structured protocol (21). Briefly, using radiation oncology treatment records, region-specific radiotherapy (RT) dosimetry, including brain RT, chest RT, and abdomen or pelvic RT, was estimated (22). Cumulative doses of individual chemotherapeutic agents, including alkylating agents, anthracyclines, epipodophyllotoxins, glucocorticoids, platinum, and vincristine, were abstracted from medical records. Equivalency approaches were applied for cumulative alkylating agent exposure (23) and anthracyclines exposure (24).

Health Behaviors

Five suboptimal health behaviors assessed by questionnaires were defined as follows: 1) less than 150 minutes per week of at least moderate physical activity (25), 2) not participating in resistance training (25), 3) current or former tobacco smoking defined as 5 or more packs during lifetime, 4) a Healthy Eating Index score in the lowest tertile based on the 2015 to 2020 Dietary Guideline for Americans (26), and 5) either no or risky alcohol drinking defined as 5 or more drinks for males or 4 or more drinks for females on at least 1 day in the past (26). To compare EAA by health behaviors, survivors were further grouped into 3 categories: 1) favorable (no or only 1 suboptimal health behavior), 2) intermediate (2 or 3 suboptimal health behaviors), and 3) unfavorable (4 or 5 suboptimal health behaviors) (27).

DNA Methylation Measurement

Genome-wide methylation data were generated using Illumina MethylationEPIC BeadChip array on whole blood-derived DNA. Genomic DNA (250 ng) was extracted from blood samples according to standard procedures as described previously (28). Further bisulfite treatment, array hybridization, and scanning were detailed in the Supplementary Methods (available online). The raw intensity data were analyzed with Illumina Genome Studio to calculate the β values, ranging from 0 to 1, to describe the methylation level of a specific CpG site. Detailed quality controls were described in the Supplementary Methods (available online).

Epigenetic Age and EAA

Epigenetic age, referred to as DNAmPhenoAge, was calculated for each participant as previously described (18). Briefly, phenotypic age was constructed from 10 physiological measurements including chronological age, and then elastic net regression analysis was employed with phenotypic age as the outcome and CpG sites as the predictors based on genome-wide

methylation data from whole-blood DNA. Finally, the selected 513 informative CpG sites were used to calculate epigenetic age with the following formula:

$$\text{DNAmPhenoAge} = \text{Intercept} + \text{CpG}_1 \times \beta_1 + \text{CpG}_2 \times \beta_2 + \dots + \text{CpG}_{513} \times \beta_{513}$$

where the intercept and coefficient values (β) of 513 CpG markers were obtained from Levine et al. (18). EAA was estimated as the residual value from a linear regression model of epigenetic age on chronological age (ie, age at DNA sampling).

Statistical Analyses

Pearson's correlation coefficient (r) was used to measure the strength of the linear association between epigenetic age and chronological age among survivors or noncancer controls. Validity of the linearity assumption was checked (Supplementary Methods, available online). To compare the annual change of epigenetic age between survivors and controls, a multiple linear regression of epigenetic age against chronological age and survivor or control status was analyzed, and the difference in the slope with respect to chronological age was tested between survivors and controls by an interaction term (chronological age $\times$ survivor or control status). To evaluate the effect of cancer diagnoses on EAA, multiple linear regression of EAA was employed adjusting for sex and health behaviors, where survivor (by primary diagnosis group) or control status was the independent variable (categorical) of interest. Adjusted least square mean (ALSM) of EAA was calculated based on this model for each primary diagnosis group and controls, and their difference was tested by Dunnett-Hsu Test, a modified Dunnett's multiple comparison test using Hsu's approximation for coverage probability estimates (29), using the control group as the reference with which each primary diagnosis group was compared. To examine effect of cancer treatments on EAA, multiple linear regression of EAA was used for each treatment (binary) adjusting for sex; those treatments with P values less than .05 were retained in a final combined model adjusting for health behaviors and sex. Difference in the ALSM of EAA between the exposed and nonexposed groups for each treatment was tested by the 2-sample t test. To evaluate the effect of health behaviors on EAA among controls, multiple linear regression was applied adjusting for sex, where health behavior status was set as the independent variable (categorical, $n=3$) and EAA was set as the dependent variable. For the survivors-only model, treatment exposures were additionally adjusted. Dunnett-Hsu test was used to compare the difference of ALSM of EAA between unfavorable or intermediate health behavior group and the favorable group. To evaluate associations between EAA and new-onset CHCs, which were defined as CHCs that occurred 180 days or more after DNA sampling, time-to-event analyses using multivariable piecewise-exponential regression were conducted (Supplementary Methods, available online). All analyses were performed with SAS 9.4 (SAS Institute, Inc, Cary, NC) and figures were generated with R 3.6.1 (30). Two-sided P less than .05 was considered as statistically significant except for the trend test (1-sided).

Results

Characteristics of Study Population

Of the 2139 survivors (Table 1), 53.0% were male and 1.1% was Hispanic. Primary diagnoses comprised leukemia (34.2%), lymphoma (21.5%), sarcoma (13.2%), central nervous system tumors

(11.5%), and other solid tumors (19.6%). Among survivors, 31.3% were exposed to brain RT, 28.5% chest RT, 22.4% abdominal or pelvic RT, 59.0% alkylating agents, 59.0% anthracyclines, 35.7% epipodophyllotoxins, 48.8% glucocorticoids, 12.7% platinum, and 69.7% vincristine. Nearly one-half of survivors (44.2%) reported participating in physical activity less than 150 minutes per week, 56.6% were not participating in resistance training, 21.8% had a poor Healthy Eating Index, 36.2% were current or former tobacco smokers, and 38.5% reported no or risky drinking history. The median ages at diagnosis, DNA sampling, and last follow-up among survivors were 7.3 years (range = 0.0 to 23.6 years), 31.8 years (range = 6.0 to 66.4 years), and 35.1 years (range = 9.2 to 69.1 years), respectively.

Among the 282 noncancer controls, 48.6% were male and 2.1% Hispanic. The median age at DNA sampling and last follow-up of controls was 35.0 years (range = 18.7 to 70.2 years) and 35.0 years (range = 18.6 to 70.2 years).

EAA in Survivors and Noncancer Controls

The estimated epigenetic age was highly correlated with chronological age among both survivors ($r=0.88$, $P<.001$) and noncancer controls ($r=0.88$, $P<.001$) (Supplementary Figure 1, available online). However, the slope of the linear regression line (ie, annual change of epigenetic age) was statistically significantly higher among survivors than controls (1.24 vs 1.08 years, $P<.001$) (Figure 1). In addition, EAA in survivors was statistically significantly higher overall (ALSM = 0.63 years, 95% CI = -0.26 to 1.01 years, $P<.001$) and across all primary diagnoses ($P<.01$) than noncancer controls (ALSM = -3.61, 95% CI = -4.43 to 2.80). Among survivors, those diagnosed with Hodgkin lymphoma had the largest EAA (ALSM = 4.61, 95% CI = 3.69 to 5.53), whereas survivors with central nervous system tumors had a relatively smaller EAA (ALSM = -1.27, 95% CI = -2.28 to -0.25) than survivors with other diagnoses (Figure 2; Supplementary Tables 1 and 2, available online).

EAA and Cancer Treatment Exposures

When comparing EAA between survivors exposed and not exposed to specific RT treatment fields or chemotherapeutic agents in univariate analyses, survivors exposed to chest RT (ALSM = 2.90 vs -1.21, $P<.001$) or abdomen or pelvic RT (ALSM = 2.98 vs -0.92, $P<.001$) had statistically significantly higher EAA than nonexposed survivors, but no association between EAA and brain RT was identified (Supplementary Table 3, available online). Survivors exposed to alkylating agents (ALSM = 1.11 vs -1.69, $P<.001$), anthracyclines (ALSM = 0.55 vs -0.89, $P<.001$), glucocorticoids (ALSM = 0.65 vs -0.70, $P<.001$), vincristine (ALSM = 0.55 vs -1.41, $P<.001$), or epipodophyllotoxins (ALSM = 0.77 vs -0.49, $P<.001$) also had higher EAA than those not exposed. However, no statistically significant association of EAA with platinum was observed (ALSM = 0.47 vs -0.12, $P=.20$) (Supplementary Table 3, available online). In addition, linear dose response for treatment and EAA associations was observed for chest RT, abdomen or pelvic RT, anthracyclines, and alkylating agents; however, risk for the highest dose group became attenuated for glucocorticoids, vincristine, and epipodophyllotoxins (Supplementary Table 4, available online). Because treatment regimens in children with cancer are often multimodal, a multivariable model including the 7 statistically significant treatments identified in the univariate model was also analyzed, showing the mutually adjusted statistical

Table 1. Characteristics of participants included in the study

Characteristics	Overall survivors No. (%)	Restricted survivors ^a No. (%)	Noncancer controls ^a No. (%)	Overall survivors vs controls ^P	Restricted survivors vs controls ^P
Total	2139	1563	282		
Sex					
Male	1133 (53.0)	825 (52.8)	137 (48.6)	.17	.19
Female	1006 (47.0)	738 (47.2)	145 (51.4)		
Ethnicity					
Hispanic	24 (1.1)	16 (1.0)	6 (2.1)	.18	.15
Non-Hispanic	2115 (98.9)	1547 (99.0)	276 (97.9)		
Diagnosis					
Leukemia	732 (34.2)	499 (31.9)			
Acute lymphoblastic leukemia	671 (31.4)	453 (29.0)	—		
Acute myeloid leukemia	59 (2.8)	44 (2.8)	—		
Other leukemia	2 (0.1)	2 (0.1)	—		
Lymphoma	460 (21.5)	347 (22.2)			
Hodgkin lymphoma	296 (13.8)	237 (15.2)	—		
Non-Hodgkin lymphoma	164 (7.7)	110 (7.0)	—		
Sarcoma	283 (13.2)	218 (13.9)	—		
Ewing sarcoma	76 (3.6)	66 (4.2)	—		
Osteosarcoma	76 (3.6)	62 (4.0)	—		
Rhabdomyosarcoma	73 (3.4)	44 (2.8)	—		
Nonrhabdomyosarcoma	58 (2.7)	46 (2.9)	—		
CNS tumors	245 (11.5)	211 (13.5)			
Astrocytoma or glioma	113 (5.3)	97 (6.2)	—		
Medulloblastoma or PNET	59 (2.8)	53 (3.4)	—		
Ependymoma	27 (1.3)	24 (1.5)	—		
Other CNS tumors	46 (2.2)	37 (2.4)	—		
Embryonal	285 (13.3)	198 (12.7)			
Wilms tumor	140 (6.5)	94 (6.0)	—		
Neuroblastoma	109 (5.1)	80 (5.1)	—		
Germ cell tumor	36 (1.7)	24 (1.5)	—		
Other	134 (6.3)	90 (5.8)			
Retinoblastoma	51 (2.4)	33 (2.1)	—		
Hepatoblastoma	15 (0.7)	10 (0.6)	—		
Melanoma	13 (0.6)	10 (0.6)	—		
Carcinomas	24 (1.1)	15 (1.0)	—		
Others	31 (1.4)	22 (1.4)	—		
Radiation					
Brain RT	669 (31.3)	456 (29.2)	—		
Chest RT	609 (28.5)	482 (30.8)	—		
Abdomen/pelvis RT	480 (22.4)	364 (23.3)	—		
Chemotherapy					
Alkylating agent	1262 (59.0)	921 (58.9)	—		
Anthracyclines	1262 (59.0)	943 (60.3)	—		
Epipodophyllotoxins	764 (35.7)	554 (35.4)	—		
Glucocorticoids	1044 (48.8)	744 (47.6)	—		
Platinum	272 (12.7)	219 (14.0)	—		
Vincristine	1491 (69.7)	1056 (67.6)	—		
Health behaviors ^b					
Physical activity				<.001	.001
≥150 min/wk	1056 (49.4)	766 (49.0)	171 (60.6)		
<150 min/wk	946 (44.2)	667 (42.7)	96 (34.0)		
Unknown	137 (6.4)	130 (8.3)	15 (5.3)		
Strength				.005	.01
Normal	777 (36.3)	557 (35.6)	129 (45.7)		
Abnormal	1211 (56.6)	872 (55.8)	139 (49.3)		
Unknown	151 (7.1)	134 (8.6)	14 (5.0)		
Health eating index				.01	.02
Normal	1496 (69.9)	1087 (69.6)	226 (80.1)		
Abnormal	467 (21.8)	328 (21.0)	46 (16.3)		
Unknown	176 (8.2)	148 (9.5)	10 (3.5)		
Tobacco smoking status				.25	.51
Never smoking	1296 (60.6)	958 (61.3)	178 (63.1)		

(continued)

Table 1. (continued)

Characteristics	Overall survivors No. (%)	Restricted survivors ^a No. (%)	Noncancer controls ^a No. (%)	Overall survivors vs controls ^P	Restricted survivors vs controls P
Ever smoking	775 (36.2)	537 (34.4)	91 (32.3)		
Unknown	68 (3.2)	68 (4.4)	13 (4.6)		
Alcohol drinking status				.01	.01
Moderate drinking	965 (45.1)	685 (43.8)	125 (44.3)		
Not moderate drinking	823 (38.5)	586 (37.5)	148 (52.5)		
Unknown	351 (16.4)	292 (18.7)	9 (3.2)		
Median age at diagnosis (range), y	7.3 (0.0, 23.6)	7.8 (0.0, 22.8)	NA		
Median age at DNA sampling (range), y	31.8 (6.0, 66.4)	31.5 (6.0, 66.4)	35.0 (18.7, 70.2)	<.001	<.001
Median age at follow-up (range), y	35.1 (9.2, 69.1)	34.1 (9.2, 69.1)	35.0 (18.6, 70.2)	.59	.26

^aSurvivors with DNA extraction conducted by the same laboratory with the same protocol as noncancer controls. CNS = central nervous system; PNET = primitive neuroectodermal tumor; RT = radiotherapy.

^bSuboptimal health behaviors included: 1) less than 150 min/wk of at least moderate physical activity, 2) no participation in resistance training, 3) scoring in the lowest tertile on the Healthy Eating Index based on the 2015 to 2020 Dietary Guideline for Americans, 4) tobacco smoking, and 5) either no or risky alcohol drinking. Individuals with missing values for health behavior variables were dropped from analyses.

Figure 1. Linear regression lines of epigenetic age by age at DNA sampling for survivors and controls.

significance in chest RT ($P = .001$), abdomen or pelvic RT ($P = .05$), alkylating agents ($P = .001$), epipodophyllotoxins ($P = .001$), and glucocorticoids ($P = .01$) (Figure 3), whereas anthracyclines and vincristine became non-statistically significant.

EAA and Health Behaviors

Among controls without a childhood cancer history, favorable, intermediate and unfavorable health behaviors were endorsed in 39.0% (101 of 259), 50.6% (131 of 259), and 10.4% (27 of 259), respectively. Among survivors, the proportion in the favorable health category (30.8%, 566 of 1838) was lower than that in noncancer controls, whereas the proportions in the intermediate (54.6%, 1003 of 1838) or unfavorable (14.6%, 269 of 1838) health behavior groups were relatively higher.

Compared with survivors with favorable health behaviors (ALSM = 0.26, 95% CI = 0.36 to 0.87), EAA was statistically significantly higher among groups of survivors with intermediate (ALSM = 1.07, 95% CI = 0.59 to 1.54, $P = .04$) or unfavorable health behaviors (ALSM = 1.45, 95% CI = 0.60 to 2.30, $P = .03$) (Figure 4, A). Among noncancer controls, EAA was also

statistically significantly higher among those with unfavorable health behaviors (ALSM = -1.23 , SE = 1.13, $P = .03$) than those with favorable health behaviors (ALSM = -4.33 , SE = 0.59) (Figure 4, B).

Analysis of EAA for specific health behaviors (Supplementary Figure 2, available online) showed that survivors with a history of tobacco smoking had statistically significantly higher EAA than those without ($P = .04$). Survivors with lower levels of physical activity ($P = .09$), poor diet ($P = .15$), or risky alcohol consumption ($P = .06$) also showed increased EAA, although these associations did not reach statistical significance.

EAA and CHCs

In time-to-event analyses (Supplementary Table 7, available online), we found evidence of associations between EAA and rates of 7 subsequent CHCs, including hypertension (third vs first tertile, relative rate [RR] = 1.83, 95% CI = 1.17 to 2.83, $P_{\text{trend}} = .005$), myocardial infarction (RR = 2.91, 95% CI = 1.27 to 7.21, $P_{\text{trend}} = .005$), obesity (RR = 1.39, 95% CI = 1.17 to 1.66, $P_{\text{trend}} < .001$), obstructive pulmonary deficit (RR = 1.86, 95% CI = 0.95 to 3.77, $P_{\text{trend}} = .05$), peripheral motor neuropathy (RR = 2.89, 95% CI = 1.24 to 6.97, $P_{\text{trend}} = .008$), peripheral sensory neuropathy (RR = 2.04, 95% CI = 0.99 to 4.26, $P_{\text{trend}} = .04$), and pulmonary diffusion deficits (RR = 2.75, 95% CI = 0.95 to 7.63, $P_{\text{trend}} = .007$) (Table 2). We also observed a borderline statistically significant association for obstructive sleep apnea with EAA (RR = 1.70, 95% CI = 0.92 to 3.02, $P_{\text{trend}} = .06$) (Table 2).

Discussion

Using DNA methylation-based epigenetic age among childhood cancer survivors and noncancer controls, we found that in contrast to the similar age-dependent attrition of LTL observed for both survivors and controls in our previous study (10), the annual change of epigenetic age is statistically significantly higher among survivors and persists across the entire age span, suggesting different modes of action underlying the dynamics of aging biomarkers (ie, LTL and epigenetic age) during the aging process: LTL follows an accentuated aging trajectory, whereas epigenetic age follows an accelerated aging trajectory (Figure 5).

Figure 2. Adjusted least square mean (ALSM) of epigenetic age acceleration (EAA) in controls and survivors overall and across primary cancer diagnoses. P values refer to the statistical significance of differences in ALSM of EAA between survivors (overall and by diagnosis) and controls. Detailed estimates are provided in the [Supplementary Table 1](#) (available online). Statistical significance for pairwise comparisons of ALSM of EAA among primary cancer diagnosis groups is provided in the [Supplementary Table 2](#) (available online). ALL = acute lymphoblastic leukemia; CNS = central nervous system; HL = Hodgkin lymphoma; NBL = neuroblastoma; NHL = non-Hodgkin lymphoma; WT = Wilms tumor.

Figure 3. Adjusted least square mean (ALSM) of epigenetic age acceleration (EAA) among survivors by cancer treatments in a multivariable model. Detailed estimates are provided in the [Supplementary Table 5](#) (available online). RT = radiotherapy.

In addition, our findings provided evidence that specific cancer treatment exposures contribute to increased levels of EAA among survivors, suggesting that cellular damage and molecular alterations induced by cancer treatments contribute to accelerated epigenetic regulation of aging. Considering the findings that unfavorable health behaviors contribute to elevated levels of EAA, which is also associated with increased incidences of age-related CHCs among survivors of childhood cancer, we propose that EAA may serve as a potential biomarker for identifying groups with higher risk and evaluating the effectiveness of pharmaceutical and behavioral interventions designed to slow down EAA and hence prevent or remediate chronic diseases in this vulnerable population.

DNA methylation is the most common epigenetic regulation mechanism, which is highly responsive to both internal and

external insults. As genotoxic treatment modalities, ionizing radiation and chemotherapeutic agents could profoundly affect the methylation level in blood leukocytes (31–34). It is reported that the oxidative stress and chronic DNA damage caused by genotoxic agents, such as radiation (35) and alkylating agents (36), could lead to aberrant local hypermethylation or global hypomethylation and ultimately engender accelerated biological aging process (37–39). However, the mechanism of how radiation and chemotherapy pose impact on EAA is still quite poorly understood. Our findings provided novel evidence that EAA is statistically significantly accelerated among adults exposed to certain cancer treatment modalities during childhood, including chest RT, abdomen or pelvic RT, alkylating agents, glucocorticoids, and epipodophyllotoxins. However, because treatment for childhood cancer includes multiple agents, we

Figure 4. Adjusted least square mean (ALSM) of epigenetic age acceleration (EAA) among survivors (A) and noncancer controls (B) by groups of health behaviors. Detailed estimated are provided in the [Supplementary Table 6](#) (available online).

Table 2. Associations of EAA with incidence of subsequent CHCs with statistical significance among survivors^a

CHC	Relative rate (95% CI)	P ^b
Hypertension		.005
First tertile	Referent	
Second tertile	0.99 (0.58 to 1.61)	
Third tertile	1.83 (1.17 to 2.83)	
Myocardial infarction		.005
First tertile	Referent	
Second tertile	1.21 (0.47 to 3.38)	
Third tertile	2.91 (1.27 to 7.21)	
Obesity		<.001
First tertile	Referent	
Second tertile	1.30 (1.09 to 1.54)	
Third tertile	1.39 (1.17 to 1.66)	
Obstructive pulmonary deficit		.05
First tertile	Referent	
Second tertile	1.26 (0.63 to 2.75)	
Third tertile	1.86 (0.95 to 3.77)	
Obstructive sleep apnea		.06
First tertile	Referent	
Second tertile	0.94 (0.49 to 1.83)	
Third tertile	1.70 (0.92 to 3.02)	
Peripheral motor neuropathy		.008
First tertile	Referent	
Second tertile	1.27 (0.46 to 3.18)	
Third tertile	2.89 (1.24 to 6.97)	
Peripheral sensory neuropathy		.04
First tertile	Referent	
Second tertile	1.14 (0.51 to 2.52)	
Third tertile	2.04 (0.99 to 4.26)	
Pulmonary diffusion deficit		.007
First tertile	Referent	
Second tertile	0.65 (0.17 to 2.49)	
Third tertile	2.75 (0.95 to 7.63)	

^aThe time-to-event analysis was performed by using a multivariable piecewise-exponential regression model for each CHC. CI = confidence interval; CHC = chronic health condition; EAA = epigenetic age acceleration.

^bP value for testing the linear trend of association with CHC across 3 tertiles of EAA.

cannot rule out entirely influences of other regimens. For example, correlations between vincristine exposure and alkylating agent or glucocorticoid exposure may have led to a non-statistically significant association between EAA and vincristine.

The age-related CHCs linked to elevated EAA in our study are consistent with previous findings. For example, positive associations of EAA with cardiovascular disease (18), hypertension (40), obesity (41), and neuropathology (18) are also reported in the general population. In addition, we provided novel evidence that links accelerated epigenetic age to pulmonary impairments among childhood cancer survivors based on the hypothesis that DNA methylation is associated with the biological process underlying lung function decline (42). Although the generation of reactive oxygen species and the increase of mitochondrial activity (43) caused by environmental exposures and suboptimal lifestyle factors (ie, smoking, poor dietary habits, excessive alcohol consumption, and inactivity) could dysregulate methylation of immune genes and increase proinflammatory responses (44) in the general population, and the chronic low-grade inflammation further fosters onset of CHCs in older individuals (45), we speculate that the process may be similar in younger childhood cancer survivors whose early treatment exposures activate this process prematurely. Thus, EAA may have utility as a biomarker that improves precision in models designed to predict CHC risk among childhood cancer survivors.

Although this study provides novel information and intriguing insights into understanding the accelerated aging among childhood cancer survivors, our findings should be interpreted in the context of some limitations. First, the low numbers of survivors with specific CHCs and matched noncancer controls affects the overall statistical power of our study. Second, because we only considered childhood cancer treatment within 5 years from initial diagnosis and additional treatment exposures during survivorship care were not considered, our results may be confounded. Third, because we had so few survivors with non-European ancestry, our analysis was restricted to

Figure 5. Dynamics of epigenetic age and telomere length differs between individuals with no history of cancer and survivors of childhood cancer due to exposures to chemotherapy and/or radiation therapy.

those of European ancestry and cannot be generalized to survivors in other ancestry groups. Fourth, the temporal association between health behaviors and EAA is uncertain, given that we do not have exact information on lifetime exposure to alcohol, poor diet, and sedentary behavior. Thus, a longitudinal multi-time point study design is needed to evaluate duration, intensity, and patterns of health behaviors over time on EAA. Fifth, although we accounted for different DNA extraction methods with batch effect adjustment in the analysis, measurement error is possible and may have influenced the results. Finally, because survivors included in this study are quite young, with a median attained age of 36 years, the estimated associations between EAA and CHC risk may change over the course of further follow-up.

Collectively, our findings provide compelling evidence that the reversible biomarker, EAA, is higher among childhood cancer survivors and possibly mediates the causal association between modifiable risk factors and age-related chronic outcomes. There is great potential for EAA to be used as a target for mechanistic antiaging interventions and also as an intermediate outcome for interventions without the need to wait for years for the endpoint to materialize.

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C.L.W. assisted in or supervised sample collection and processing; J.E., H.M., E.P., G.N., E.W., and J.Z. supervised and/or performed DNA extraction and processing for the Infinium MethylationEPIC Array. N.Q. and Z.W. performed the methylation analysis. Y.Y., Z.W., and L.L.R. developed the statistical analysis plan. Z.L. and N.Q. performed the statistical analyses. N.Q., N.S., and Z.W. wrote the first draft of the manuscript. All authors contributed to data interpretation and writing and approved the final manuscript for publication.

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Data availability

The DNA methylation data is accessible through the St Jude Cloud (<https://stjude.cloud>).

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