Vasomotor symptoms and accelerated epigenetic aging in the Women's Health Initiative (WHI)

Rebecca C. Thurston, PhD[a]

Judith E. Carroll, PhD[b]

Morgan Levine, PhD[c]

Yuefang Chang, PhD[d]

Carolyn Crandall, MD, MS[e]

JoAnn E. Manson, MD, DrPH[f]

Lubna Pal, MBBS[g]

Lifang Hou, MD, PhD[h]

Aladdin H. Shadyab, PhD[i]

Steve Horvath, PhD[j]

[a]Departments of Psychiatry and Epidemiology, University of Pittsburgh School of Medicine

[b]Cousins Center for Psychoneuroimmunology, Department of Psychiatry and Biobehavioral Sciences, Semel Institute for Neuroscience and Human Behavior, University of California at Los Angeles School of Medicine

[c]Department of Pathology, Yale School of Medicine

[d]Department of Neurosurgery, University of Pittsburgh School of Medicine

[e]Department of Medicine, David Geffen School of Medicine at the University of California at Los Angeles School of Medicine

[f]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School

[g]Department of Obstetrics, Gynecology & Reproductive Sciences, Yale School of Medicine

[h]Department of Preventive Medicine, Northwestern Feinberg School of Medicine

[i]Department of Family Medicine and Public Health, University of California, San Diego School of Medicine, La Jolla, CA

[j]Department of Biostatistics, University of California at Los Angeles

Corresponding author:

Rebecca C. Thurston, PhD, University of Pittsburgh, 3811 O'Hara St, Pittsburgh, PA 15213; 412-648-9087; 412-648-7160 (fax); thurstonrc@upmc.edu

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Abstract

Purpose: The hallmark menopausal symptom, vasomotor symptoms (VMS), has been linked to adverse health indicators. However, the relationship between VMS and biological aging has not been tested. We examined associations between menopausal VMS and biological aging as assessed by two DNA methylation-based epigenetic aging indicators previously linked to poor health outcomes.

Methods: Participants were members of the Women's Health Initiative Observational Study (WHI-OS) integrative genomics sub-study (N = 1,206) who had both ovaries and were not taking hormone therapy. Relationships between VMS at enrollment (presence, severity) or VMS timing groups (no VMS: not at menopause onset nor at study enrollment; early VMS: at menopause onset but not at enrollment; persistent VMS: at menopause onset and study enrollment; and late VMS: at enrollment but not at menopause onset) and epigenetic clock indicators predictive of physical aging and early death (DNAm PhenoAge, DNAm GrimAge) were tested in linear regression models adjusting for age, race/ethnicity, hysterectomy, education, body mass index, smoking, and in additional models, sleep disturbance.

Results: Women were on average 65 years of age at enrollment. Severe hot flashes at enrollment were associated with higher DNAm PhenoAge [relative to no hot flashes: B(SE)=2.79(1.27), p=.028, multivariable]. Further, late-occurring VMS were associated with both higher DNAm PhenoAge [B(SE)=2.15 (.84), p=.011] and DNAm GrimAge [B(SE)=1.09 (.42), p=.010, multivariable] relative to no VMS.

Main Conclusions: Among postmenopausal women, severe or late-occurring VMS was associated with accelerated epigenetic age, controlling for chronological age. Postmenopausal women with severe or late-occurring VMS may have greater underlying epigenetic aging.

Key Words: vasomotor symptoms, hot flashes, menopause, epigenetics, biologic aging, aging

Précis Among 1206 members of the Women's Health Initiative Observational Study Integrative Genomics Study, women with severe or late-occurring vasomotor symptoms had evidence of accelerated epigenetic aging.

Introduction

The menopause is a universal transition experienced by all women living into midlife. Vasomotor symptoms (VMS, including hot flashes and night sweats) are the hallmark of menopause. While most women will experience VMS, in a minority of women, VMS will be severe.[1] Whereas traditionally, VMS are thought to largely occur within the few years around the onset of menopause, newer information indicates that VMS follow distinct patterns during the menopausal transition and into post-menopause. In fact, for some women, VMS begin only after they become menopausal, and can persist for several years.[2] VMS have implications for women's health and functioning, including poorer sleep,[3] mood,[4] and overall impairments in social, emotional, and physical quality of life.[5] Newer data also indicate that VMS may be associated with a range of future adverse physical health outcomes (e.g., cardiovascular disease (CVD) risk,[6-8] diabetes,[9] hypertension,[10] and lower bone density[11]).

The menopause transition is a period of reproductive aging that occurs directly before the onset of many chronic diseases. Multiple health indices have been shown to worsen during the menopause beyond chronologic aging alone[12]; thus the menopause can be conceptualized as a time of accelerated biologic aging. In fact, in prior work, aspects of the menopause (e.g., earlier menopause, longer time since menopause) have been linked to accelerated epigenetic aging, an index of biologic aging, beyond the effects of chronologic aging alone.[13] Other work has indicated that women reporting more frequent and more types of insomnia symptoms, a common menopausal experience, exhibited greater epigenetic aging.[14] These studies leveraged DNA methylation (DNAm)-based epigenetic clock indicators, which may capture aspects of biological aging beyond chronological age alone. No prior work has examined the relation between menopausal VMS and epigenetic aging.

We test the hypothesis that bothersome hot flashes and night sweats (aka, VMS) are associated with accelerated epigenetic aging. Given the importance of late-occurring VMS to major health outcomes (e.g., CVD) in the WHI,[8] we also examine the timing of VMS in relation

to epigenetic aging, and hypothesize that late-occurring VMS will be those most associated with epigenetic aging. Our primary epigenetic aging indicators are DNAm PhenoAge and DNAm GimAge, as these are most closely tied to phenotypic aging and future adverse health outcomes (e.g., cancers, decreased physical functioning, shorter health span, Alzheimer's disease, coronary heart disease, and all-cause mortality[15,16]). In additional models, we examine any role of sleep disturbance in these relationships as well as relationships between VMS and additional epigenetic aging indices (Hannum[17] and Horvath[18]).

Methods

Participants

The study sample was comprised of women who participated in the WHI Observational Study (WHI-OS). Details of the WHI-OS have been published previously[19,20] and data are publicly available via dbGaP. Women were eligible to participate in the WHI-OS if they were 50–79 years of age, were postmenopausal, were willing to provide written informed consent, and resided in a nearby area within proximity of 40 WHI clinical centers across the United States. Recruitment for the baseline assessment occurred from 1993 to 1998. The current analyses were based on the WHI subsample (N=2107) who participated in an integrative genomics study, which oversampled African American and Hispanic women.[21] Women were excluded from the current analysis if they had missing VMS data (N=13), had undergone oophorectomy (N=700), or were taking hormone therapy (HT) at the time of the VMS assessment of interest given the pronounced impact of HT on VMS (200 women who reported current HT at enrollment were excluded from analyses of VMS at enrollment, and an additional 245 women who reported past HT use were additionally excluded from analyses that examined past VMS in conjunction with VMS at enrollment). The final analytic samples were comprised of 1,194 and 949 women for analyses of VMS at enrollment and VMS history, respectively.

Vasomotor Symptoms (VMS)

Women completed questionnaires at enrollment that queried the presence of (in the prior four weeks) and bothersome nature of VMS (hot flashes and night sweats; none, mild, moderate, severe). Women were also asked about past VMS and the age at which their VMS first occurred. As previously described,[8] using VMS data in conjunction with reported age at menopause, women were classified into one of four groups: early VMS (at the time of menopause onset only), late VMS (at study enrollment), persistent VMS (VMS both at the time of menopause onset and study enrollment), and no VMS (no VMS at enrollment nor at the time of menopause).

DNAm Profiling

Methylation analyses were performed from samples drawn from the baseline WHI visit at HudsonAlpha Institute of Biotechnology (Huntsville, AL) using the Illumina Infinium HumanMethylation450 BeadChip (Illumina, Inc., San Diego, CA), which as previously described[21] includes 485,577 different CpG sites.

DNAm Age Indices

DNAm PhenoAge. The development and validation of the epigenetic biomarker of phenotypic age, DNAm PhenoAge, has been detailed elsewhere.[15] Briefly, an estimate of clinical phenotypic age was developed using the NHANES III as training data, in which a proportional hazards penalized regression model was employed to narrow 42 biomarkers to 9 biomarkers and chronological age; this was subsequently validated in NHANES IV. Next, DNAm PhenoAge was developed by regressing phenotypic age on blood DNA methylation data using the InCHIANTI data, which produced an estimate of DNAm PhenoAge based on a weighted sum of 513 CpGs. This measure was subsequently validated using multiple cohorts, aging-related outcomes, and tissues/cells. Finally, the underlying biology of the 513 CpGs and the

DNAm PhenoAge measure was examined using a variety of complementary data and various genome annotation tools including chromatin state analysis and gene ontology enrichment. In general, DNAm PhenoAge can be contrasted against chronological age to infer accelerated/decelerated aging. Individuals whose DNAm PhenoAge exceeds their chronological age are thought to be aging at an accelerated rate, which is in line with findings that these individuals tend to have higher mortality and morbidity risk.

DNAm GrimAge The development and validation of the epigenetic biomarker DNAm GrimAge has been detailed elsewhere.[16] Briefly, in our previous publication, the Framingham Heart Study Offspring Cohort was initially used as training data to define DNAm-based surrogate markers of 88 plasma protein variables and smoking pack-years. Each of the 88 plasma protein variables was regressed on chronological age, sex, and the CpG levels in the training data using an elastic net regression model. The number of proteins was then narrowed to 12, which represented those shown to have a moderately high correlation between measured plasma levels and the respective DNAm-based surrogate marker in the test data set. We then developed a single composite biomarker of lifespan, DNAm GrimAge, by regressing time-to-death due to all-cause mortality on the 12 DNAm-based surrogate biomarkers of plasma protein levels, a DNAm-based estimator of smoking pack-years, chronological age, and sex. A large-scale meta-analysis (involving more than 7000 Illumina array measurements from 6,935 participants in four cohort studies), was then used to validate DNAm GrimAge, showing that its ability to predict lifespan far exceeded predictions based on other DNAm-based aging measures. As with DNAm PhenoAge, higher values indicate greater epigenetic aging.

Additional epigenetic indices. Hannum and Horvath indices and their implementation in the WHI have been described in detail previously.[14] Briefly, the Hannum measure of extrinsic epigenetic age acceleration (EEAA) is a measure highly correlated with immune senescence.[17] This measure relies on 71 CpGs and is enhanced by forming a weighted average of this with estimated blood cell counts using the approach of Klemera and Doubal[22] from three blood cell

types that are known to change with age: naive (CD45RA+CCR7+) cytotoxic T cells, late differentiated (CD28–CD45RA–) cytotoxic T cells, and plasma B cells. The weights used in the weighted average are determined by the correlation between the respective variables and chronological age in the WHI.[22] The Horvath measure of intrinsic epigenetic age acceleration (IEAA) was estimated via the Horvath[18] method using 353 CpGs and coefficient values. In contrast to EEAA, this measure removes the variance attributable to estimated blood cell counts (naive cytotoxic T cells and late differentiated cytotoxic T cells and plasma B cells), making it a cell independent estimate of biological age. Higher values indicate greater epigenetic aging.

Covariates

Anthropometric measures were obtained at the initial screening visit. Body mass index (BMI) was calculated as weight (kg) / height (m^2). Participants completed questionnaires that inquired about age, race/ethnicity, educational attainment, and medical, reproductive, and health behavior histories (including history of hysterectomy, oophorectomy, pack years of smoking history). Participants completed a questionnaire about both current and past HT use (HT use defined as use of an estrogen- or progestogen-containing pill or transdermal patch). Insomnia symptoms were assessed by the WHI Insomnia Rating Scale (WHIIRS).[23] Five items (0–4) from the scale are summed to produce an overall global score (range: 0-20). WHIIRS scores of >10 indicate a significant sleep disturbance.[24] We also considered the total number of insomnia symptoms endorsed [≥1-2 or more times/week; to yield a sum of 0-5 insomnia symptoms], consistent with prior work.[14]

Data Analysis

All variables were examined for deviation from normality and cell sizes. Univariate relations between study variables and epigenetic aging were examined in correlations, t-tests, and ANOVA. Relationships between VMS and epigenetic aging were examined in linear

regression models. VMS predictors included (a) VMS severity at enrollment, with hot flashes and night sweats considered separately, and (b) VMS timing groups (none, early, late, and persistent).[8] Both crude and adjusted models of relations between VMS and epigenetic aging were estimated, with covariates selected based upon their relation to the epigenetic aging outcome at p<0.05 (race/ethnicity, education, BMI, smoking, hysterectomy). Chronological age was included in all models. Sleep disturbance (WHIIRS >10) or number of insomnia symptoms were added to multivariable models and considered as effect modifiers, with interactions between VMS predictors and sleep examined via cross product terms. Primary epigenetic aging outcomes were DNAm PhenoAge and DNAm GrimAge. Hannum and Horvath indices were considered as outcomes in additional models. All models were two-tailed with P<0.05 considered as statically significant. All analyses were conducted with Statistical Analysis Software Version 9.4 (SAS Institute, Cary, NC).

Results

The sample was comprised of non-Hispanic White women (55%), non-Hispanic Black women (26%), and Hispanic women (19%; Table 1). Approximately a third of the sample reported VMS at enrollment (mean [SD] age 65.1 [7.1]), and 60% of the women had VMS at some point during the menopause transition and/or at enrollment. Factors associated with greater epigenetic aging (DNAm GrimAge, DNAm PhenoAge) were racial/ethnic minority status (relative to white; p=.0001), lower education (p=.001), higher BMI (p<.0001), and for DNAm GrimAge only, greater pack years of smoking (p<.0001).

We examined associations between VMS and epigenetic aging (DNAm PhenoAge, DNAm GrimAge). Relative to no hot flashes at enrollment, severe hot flashes, but not night sweats, at enrollment were associated with higher DNAm PhenoAge, indicating greater phenotypic epigenetic aging (Table 2). When examining VMS timing groups, late VMS (but not early or persistent VMS) were associated with higher DNAm PhenoAge and DNAm GrimAge

relative to no VMS. Associations persisted when adjusting for covariates, including age.

In sensitivity analyse, we examined sleep disturbance, and found that it neither accounted for relations between VMS and epigenetic aging (Table 3), nor did it modify relations between VMS and epigenetic aging indices (p-values for interactions>.18-.46). Associations were similar when we considered total number of insomnia symptoms instead of sleep disturbance (data not shown). Further, relationships between VMS and epigenetic aging were similar when we additionally adjusted for thyroid disorder in multivariable models (data not shown). Finally, associations between VMS and Hannum and Horvath indices of epigenetic aging were not significant (data not shown).

Discussion

In this well-characterized sample of postmenopausal women, those with severe hot flashes at enrollment or late-occurring VMS showed accelerated epigenetic aging, even after accounting for chronological age. These relationships persisted when controlling for factors such body mass index, race/ethnicity, education, and sleep disturbance. These data suggest that women with severe or late-occurring VMS may be experiencing greater underlying epigenetic aging.

VMS are well-established to have implications for women's health and functioning.[3-5] However, newer data have linked VMS to several adverse health indicators, such as CVD risk,[6-8] diabetes,[9] hypertension,[10] lower bone density and higher bone turnover.[11,25] Our data build upon this body of work, suggesting that severe VMS may also mark underlying biological aging in the epigenome. Further, we found that the timing of VMS was also associated with greater epigenetic aging. Notably, the menopause transition is a dynamic period of life in which the timing of events, including VMS, can be critical to their links to health.[26-28] Newer data indicate that some women experience VMS early in the transition, largely when they are still

menstruating, and other women experience VMS later, after their final menstrual period, and many experience prolonged duration of symptoms.[2] In this investigation, only late-occurring VMS was associated with epigenetic aging. Our finding parallels prior WHI findings that late-occurring VMS are associated with CVD risk.[8] However, this pattern is not universally observed.[9,29-31] The reasons for this diverse set of observations regarding VMS timing and their associations with health outcomes is not entirely clear; however, the nature of the outcome (e.g., subclinical CVD or CVD risk factors versus clinical CVD), the study design (e.g., cross sectional, longitudinal), and the method of VMS assessment (retrospective reports, prospective reports, physiologic assessments) are likely important. To better understand the role of timing in relationships between VMS and indicators of biological aging and disease, a critical next step is to employ a prospective research design with repeated measurements of VMS, hormonal parameters, and biological age prior to onset and during the menopause transition. Our findings underscore the potential importance of VMS in biological aging of the epigenome that should be investigated in future work.

Multiple metrics have been proposed to characterize biological aging, with no one metric universally accepted. However, DNAm-based indicators have the advantage of serving as a broad-based marker of accelerated aging across a diverse set of bodily systems. Further, these epigenetic clocks, particularly recent formulations (e.g., DNAm PhenoAge, DNAm GrimAge), show robust relationships with adverse health outcomes including CVD, mortality, and Alzheimer's disease.[15,16] As these diseases have been linked to VMS, DNAm-based biological aging indices are particularly pertinent to the present research question. We found less robust associations of VMS with epigenetic clock indices that were developed to predict chronological age (i.e., IEAA), in contrast with those trained with intermediate plasma/serum biomarkers (i.e., PhenoAge, GrimAge). Our findings lend support to the utility of these later epigenetic age markers for tracking biological aging.

We considered several explanatory factors in these relationships. We considered

chronologic age, race/ethnicity, adiposity, educational attainment, and smoking, yet none of these factors explained observed associations. Although the etiology of VMS is not fully understood, changes in reproductive hormones including declines in endogenous estradiol are understood to be permissive to VMS[32] (albeit not solely explanatory) and are linked to a range of age-related diseases.[33] We were unable to consider the role of these endogenous hormones as these data were not collected in most of women in this subcohort; future work should consider the role of endogenous reproductive hormones in associations between VMS and epigenetic aging. As VMS are a consistent predictor of poor sleep as women age, and insomnia symptoms have been linked to accelerated epigenetic aging in the WHI, we considered insomnia symptoms in these relations. However, insomnia symptoms / sleep disturbance did not explain the relationship between VMS and epigenetic aging, nor was there evidence of a synergy between VMS and sleep disturbance in relation to epigenetic aging. These data suggest that VMS themselves may be linked to accelerated epigenetic aging independent of sleep.

Several limitations should be considered. Women reported VMS both at enrollment and as recalled years earlier. Memory and reporting factors can impact the accuracy of these VMS reports, particularly for recalled reports; future studies examining women prospectively from prior to onset and over the menopause transition that examine incidence of VMS are needed. Although there was substantial variation in the age of women at enrollment, the average age of the enrollees (65 years) was substantially older than the age at which VMS peak. As these are observational data and the study was cross-sectional, the directionality or causality of these associations cannot be established.

This study has several strengths. We tested a novel question in a large, well-characterized sample of women. The sample was racially/ethnically diverse, with substantial numbers of Black and Hispanic women represented. Epigenetic aging was characterized via state-of-the-art methods, and multiple potentially confounding or explanatory factors were

considered. The nature of the VMS reports allowed for an examination VMS occurrence, severity, and timing in relation to epigenetic aging.

This study indicated that postmenopausal women with severe or late-occurring VMS had greater biological aging, suggesting that VMS may mark a pattern of accelerating biological aging after menopause not explained by chronological age. These findings continue to challenge the notion of VMS as solely incidental midlife symptoms, indicating that VMS, particularly if severe or late-occurring, may mark important information about women's health and aging. Future work should consider the role of epigenetic aging in links between VMS and potential disease risk.

Data Availability: The WHI data are available through dbGAP:

http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000200.v10.p3

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Table 1. Participant Characteristics at Enrollment (N=1,194)

Table 2. Relations between VMS and epigenetic aging

†p<0.10; *p<0.05

Covariates: Age, race/ethnicity, hysterectomy, education, BMI, and (for DNAm GimAge models), smoking pack years

Note: Comparisons relative to no VMS

Table 3. Relations between VMS and epigenetic aging, controlling for sleep disturbance

†p<.10; *p<.05; **p<.01

Covariates: Age, race/ethnicity, education, BMI, hysterectomy, (for DNAm GimAge models) smoking pack years, sleep disturbance

Note: Comparisons relative to no VMS

Table 1

Age, years, M (SD)	65.13 (7.08)
Race/ethnicity, N (%)	
White	662 (55.44)
Black	310 (25.96)
Hispanic	222 (18.59)
Education, N (%)	
High school or less	539 (45.68)
College graduate	401 (33.98)
Greater than college	240 (20.34)
BMI, M (SD)	29.69 (6.24)
Hysterectomy, yes, N (%)	276 (23.12)
Sleep disturbance (WHI Insomnia Rating Scale, high, ≥10), N (%)	269 (23.21)
Years since menopause, M (SD)	17.08 (9.44)
Smoking pack years, N (%)	
0	646 (55.69)
0-≤10	205 (17.67)
>10	309 (26.64)
Hot flashes at enrollment, N (%)	
None	842 (70.94)
Mild	221 (18.62)
Moderate	96 (8.09)
Severe	28 (2.36)

Night sweats at enrollment, N (%)	
None	784 (66.27)
Mild	280 (23.67)
Moderate	94 (7.95)
Severe	25 (2.11)
VMS timing group, N (%)	
None	407 (39.82)
Early	221 (21.62)
Late	113 (11.06)
Persistent	281 (27.50)

Table 2

		DNA Phenotypic Aging	DNAm GimAge
		B (SE)	*B (SE)*
Hot Flashes at Enrollment	*Mild*	-.30 (.52)	-.30 (.27)
	Moderate	-.21 (.75)	.48 (.38)
	Severe	2.79 (1.27)*	.47 (.66)
Night Sweats at Enrollment	*Mild*	.29 (.47)	.27 (.24)
	Moderate	.93 (.74)	.37 (.38)
	Severe	-.70 (1.33)	1.27 (.67)†
VMS Timing	*Early*	.60 (.62)	-.41 (.31)
	Late	2.15 (.84)*	1.09 (.42)*
	Persistent	.80 (.62)	-.06 (.31)

Table 3

		DNA Phenotypic Aging	DNAm GimAge
		B (SE)	*B (SE)*
Hot Flashes at Enrollment	*Mild*	-.34 (.54)	-.42 (.27)
	Moderate	-.47 (.79)	.36 (.39)
	Severe	2.86 (1.39)*	.27 (.70)
Night Sweats at Enrollment	*Mild*	.22 (.50)	.20 (.25)
	Moderate	.90 (.79)	.29 (.39)
	Severe	-.79 (1.36)	1.26 (.68)†
VMS Timing	*Early*	.76 (.65)	-.33 (.32)
	Late	1.84 (.88)*	1.08 (.43)*
	Persistent	.75 (.65)	-.23 (.32)