Epigenetic Aging and Immune Senescence in Women With Insomnia Symptoms: Findings from the Women's Health Initiative Study

Supplemental Information

Supplemental Methods

Cell Subsets: CD8+, CD4+, NK Cells, B Cells, and Granulocytes

In addition to the estimation of naïve and late differentiated T cells (1), we also used the Houseman's estimation method (2), which is based on DNA methylation signatures from purified leukocyte samples, to estimate the proportions of cytotoxic (CD8+) T cells, helper (CD4+) T cells, natural killer cells, B cells, and granulocytes (see Supplemental Table S2). The software does not allow us to identify the type of granulocytes in blood (neutrophil, eosinophil, or basophil) but we note that neutrophils tend to be the most abundant granulocyte (~60% of all blood cells compared with 0.5-2.5% for eosinophils and basophils). Further analyses of sleep parameters with these estimates can be found in Supplemental Table S2.

Intrinsic Epigenetic Age Acceleration

The intrinsic epigenetic age acceleration (*IEAA*) measure uses the Horvath (2013) method, using 353 CpGs and coefficient values, as reported previously (3), to define DNAm age. These CpGs and coefficient values were chosen in independent data sets by regressing age on CpGs using the elastic net penalized regression model (implemented in the R package glmnet) (4), which does not require use of the penalty function and avoids the related limitations. The DNAm age predicted from this model is robust with respect to technical variation and is highly reliable across tissues in predicting chronological age (3), with further details on the mathematical algorithms in Horvath (1,3) and online at (https://labs.genetics.ucla.edu/horvath/dnamage/). In order to account for confounding due to blood cell composition, we also adjust for imputed

measures of blood cell counts: naive cytotoxic T cells and late differentiated cytotoxic T cells and plasma B cells. Supplemental Table S3 and Figure S1 report on the interrelationship between the IEAA and EEAA measures, along with imputed measures of cell counts.

Table S1. Linear effect model coefficient (B) and standard error (SE) for each sleep characteristic predicting intrinsic epigenetic age acceleration (IEAA).

	Model 1[a]		Model 2[a]	
Independent Predictor	**B(SE)**	***P* value**	**B(SE)**	***P* value**
Sleep Duration				
Short Sleep (< 6 hours)	-.62(.34)	.07	-.62(.34)	.07
Normal Sleep (7-8 hours; Reference)	REF	REF	REF	REF
Long Sleep (> 8 hours)	-.33(.56)	.55	-.32(.56)	.57
Sleep Disturbance (WHIIRS >10 vs 10 or less)	.31(.28)	.27	.23(.28)	.42
Wake at night (Yes vs. No)	.41(.27)	.13	.36(.27)	.18
Restless	.13(.30)	.67	.12(.30)	.69
Trouble Falling Asleep	-.13(.25)	.62	-.16(.25)	.54
Waking too early	.12(.23)	.61	.10(.23)	.65
Trouble going back to sleep	-.10(.24)	.67	-.11(.24)	.64
Any Insomnia Symptoms (Yes vs. No)	.16(.29)	.59	.09(.29)	.75

[a] Each independent predictor is entered in a separate model with IEAA as the dependent variable. Model 1 adjusts for race (Black vs. non-Black; Hispanic vs. non-Hispanic), education (category), BMI (category), and snore (yes=1). Model 2 adjusts for comorbid chronic conditions: diabetes, hypertension, and CVD.

Table S2. Point-biserial correlations of sleep parameters with the estimated proportion of cell subsets in the leukocyte pool derived from DNA methylation signatures.

	CD8		CD4		NK		B Cell		Monocytes		Granulocytes		Plasma	
	r	***P***	***r***	***P***	***r***	***P***	***r***	***P***	***r***	***P***	***r***	***P***	***r***	***P***
Short Sleep Duration	0.08	0.002	-0.01	0.85	-0.02	0.46	0.04	0.13	0.001	0.97	-0.02	0.56	-0.03	0.31
Long Sleep Duration	0.05	0.07	-0.04	0.22	-0.04	0.22	-0.04	0.20	0.01	0.66	0.03	0.34	0.00	0.99
WHIIRS (>10)	0.00	0.87	-0.04	0.09	0.00	0.83	-0.04	0.10	-0.01	0.77	0.04	0.08	0.04	0.11
Trouble Falling Asleep	-0.01	0.59	-0.01	0.60	0.00	0.96	-0.02	0.50	0.00	0.89	0.02	0.42	0.02	0.29
Waking at Night	0.02	0.49	-0.05	0.03	0.03	0.17	**-0.05**	**0.02**	-0.01	0.62	0.03	0.21	0.03	0.20
Waking too early	0.00	0.86	-0.01	0.67	-0.01	0.56	-0.03	0.13	-0.02	0.44	0.03	0.25	0.02	0.39
Trouble going back to sleep	0.02	0.33	-0.03	0.19	0.01	0.54	-0.03	0.18	0.01	0.76	0.01	0.52	0.01	0.77
Restless Sleep	0.00	0.99	0.00	0.94	0.01	0.50	-0.02	0.37	-0.02	0.50	0.01	0.54	0.02	0.29
Any Insomnia Symptom	0.01	0.52	**-0.06**	**0.01**	0.02	0.28	**-0.06**	**0.01**	-0.02	0.49	0.04	0.09	0.03	0.13

Table S3. Correlation between measures of epigenetic age, naïve CD8, and late differentiated CD8 cells.

	EEAA		IEAA		Naïve CD8+		CD8+CD28-CD45RA-	
	r	***p***	***r***	***p***	***r***	***p***	***r***	***p***
EEAA	--	---	.37	.000	.41**	.000	-.53**	.000
IEAA			---	---	.003	.91	-.004	.86
Naïve CD8+					---	---	-.50**	.000
CD8+CD28-CD45RA-							---	---

**P<.001

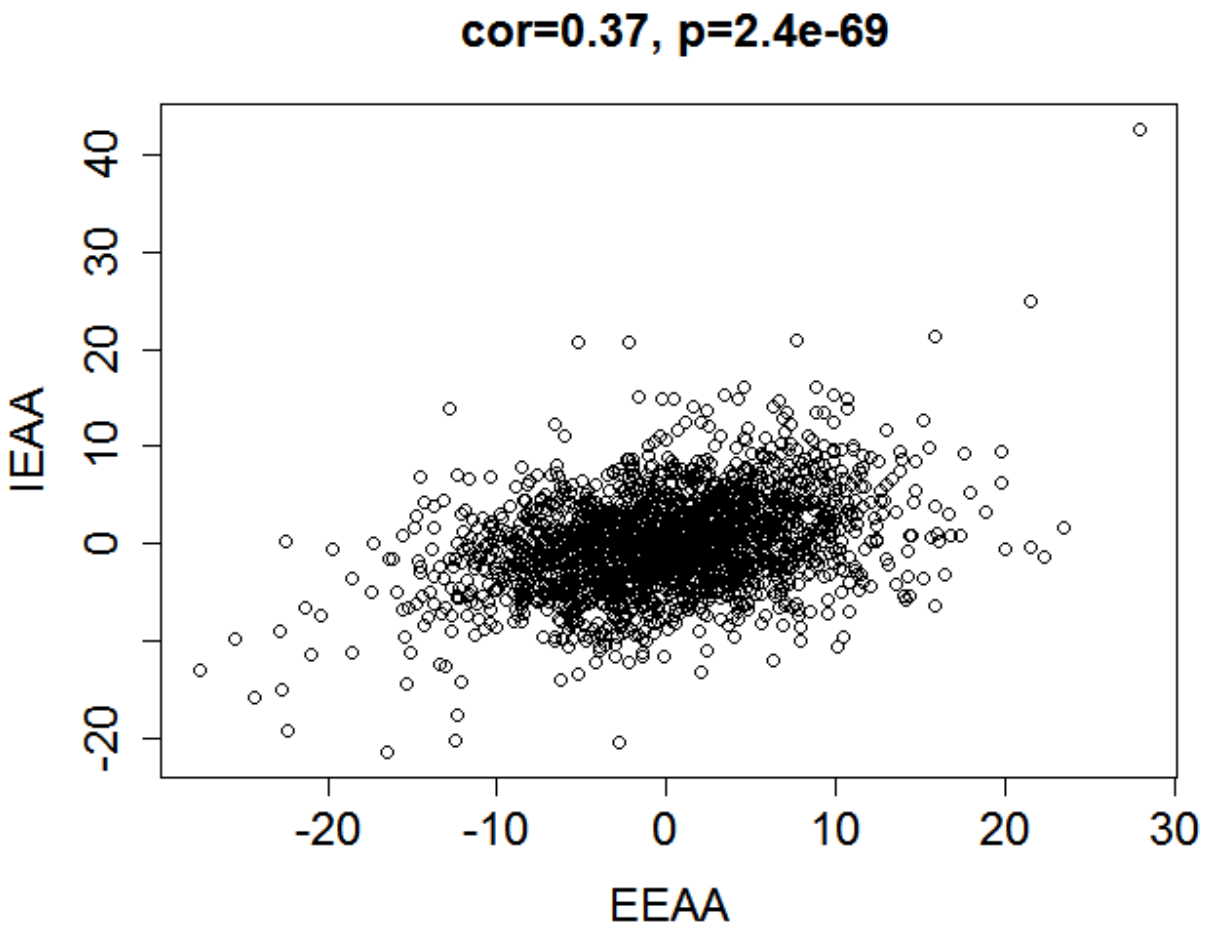

Figure S1. Scatterplot of extrinsic epigenetic age acceleration (EEAA) with intrinsic epigenetic age acceleration (IEAA).

Supplemental References

1. Horvath S, & Levine AJ (2015): HIV-1 infection accelerates age according to the epigenetic clock. *The Journal of Infectious Diseases*, 212(10), 1563–73.

2. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, *et al.* (2012): DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics*. 13: 86.

3. Horvath S (2013): DNA methylation age of human tissues and cell types. *Genome Biol*. 14: R115.

4. Friedman J, Hastie T, Tibshirani R (2010): Regularization paths for generalized linear models via coordinate descent. *J Stat Softw*. 33: 1–22