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DNA methylation age is not accelerated in brain or blood of subjects with schizophrenia

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ABSTRACT

Individuals with schizophrenia (**SZ**) exhibit multiple premature age-related phenotypes and die ~20 years prematurely. The accelerated aging hypothesis of SZ has been advanced to explain these observations, it posits that SZ-associated factors accelerate the progressive biological changes associated with normal aging. Testing the hypothesis has been limited by the absence of robust, meaningful, and multi-tissue measures of biological age. Recently, a method was described in which DNA methylation (**DNAm**) levels at 353 genomic sites are used to produce "DNAm age", an estimate of biological age with advantages over existing measures. We used this method and 3 publicly-available DNAm datasets, 1 from brain and 2 from blood, to test the hypothesis. The brain dataset was composed of data from the dorsolateral prefrontal cortex of 232 non-psychiatric control (**NPC**) and 195 SZ subjects. Blood dataset #1 was composed of data from whole blood of 304 NPC and 332 SZ subjects, and blood dataset #2 was composed of data from whole blood of 405 NPC and 260 SZ subjects. DNAm age and chronological age correlated strongly ($r = 0.92–0.95$, $p < 0.0001$) in both NPC and SZ subjects in all 3 datasets. DNAm age acceleration did not differ between NPC and SZ subjects in the brain dataset ($t = 0.52$, $p = 0.60$), blood dataset #1 ($t = 1.51$, $p = 0.13$), or blood dataset #2 ($t = 0.93$, $p = 0.35$). Consistent with our previous findings from a smaller study of postmortem brains, our findings suggest there is no acceleration of brain or blood aging in SZ and, thus, do not support the accelerated aging hypothesis of SZ.

1. Introduction

Schizophrenia (**SZ**) is associated with premature age-related phenotypes throughout the body. For example, the brains of individuals with SZ exhibit alterations that are frequently associated with old age including dendritic spine loss (Glausier and Lewis, 2013; Moyer et al., 2015), cerebral cortical atrophy (van Haren et al., 2011), and cognitive dysfunction (Jeste et al., 2011). Premature age-related phenotypes including telomere shortening (Darrow et al., 2016; Wolkowitz et al., 2016), increased inflammatory markers (Lee et al., 2017), and elevated levels of oxidative stress (Lee et al., 2016; Okusaga, 2014) have been measured from blood of SZ subjects and suggest the involvement of multiple organ systems.

Individuals with SZ also die ~20 years prematurely (Laursen et al., 2014). Rates of suicide, homicide, and accidental death are increased among individuals with SZ but most of the excess mortality has been attributed to natural causes such a cardiovascular and respiratory disease (Saha et al., 2007). Both endogenous (e.g., polygenic risk) and environmental factors (e.g., health behaviors and health care access) are thought to contribute to premature mortality in SZ. Tobacco smoking (Brown et al., 1999), sedentary lifestyle (Osborn et al., 2007), obesity (Allison et al., 1999), insulin resistance (Greenhalgh et al., 2017; Pillinger et al., 2017), and hyperlipidemia (Henderson et al., 2015) are all more common in SZ than in the general population.

The accelerated aging hypothesis of SZ has been advanced to explain these observations, it posits that SZ-associated factors, either endogenous or environmental, accelerate the progressive biological changes of normal aging (Kirkpatrick et al., 2008). Testing this hypothesis has been challenging due the absence of robust and meaningful measures of biological age across multiple cell and tissue types. However, a recently described DNA methylation (**DNAm**)-based method of measuring biological age offers promise for addressing this challenge (Horvath, 2013). By combining DNAm levels at 353 genomic sites, the method produces "DNAm age", a measure of biological age. This method estimates chronological age for healthy individuals with unprecedented accuracy, and this estimate is consistent across most cell and tissue types (Horvath, 2013). Importantly, DNAm age appears to capture an aspect of biological age as demonstrated by the fact that the difference

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between an individual's DNAm age and chronological age is associated with clinically meaningful outcomes. For example, individuals exhibiting age acceleration (i.e., DNAm age > chronological age) are at greater risk for all-cause mortality (Chen et al., 2016; Marioni et al., 2015).

Given the advantages of the above DNAm-based method of measuring biological age, we previously used it to test the accelerated aging hypothesis of SZ in the superior temporal gyrus (**STG**), a region affected by cortical atrophy and dendritic spine loss in SZ (Shelton et al., 2015; Sun et al., 2009; Sweet et al., 2009). We found age acceleration did not differ between non-psychiatric control (**NPC**) and SZ subjects. That study, however, was limited in that we studied only a single brain region in a small cohort ($N = 44$). Here, we follow-up on that study by using the same approach in 3 large, publicly-available DNAm datasets—one from brain and two from blood.

2. Experimental methods

2.1. Dataset selection and description

The datasets were chosen such that one measured DNAm in a brain region distinct from that which we studied previously and the other two measured DNAm in a peripheral tissue. Additional criteria for selecting the datasets for analysis included that they i) were publicly available, ii) measured DNAm using Human Methylation 450K array platform (**HM450K Array**; Illumina, San Diego, CA, USA), iii) included an age range of at least 30 years, and iv) made up of a relatively large number of samples compared to other similar datasets.

2.1.1. Brain dataset

These data were generated by Jaffe and colleagues (Jaffe et al., 2016) using DLPFC, defined as the middle one-third of the middle frontal gyrus immediately anterior to the genu of the corpus callosum, DNA from postmortem brains. They extracted DNA from DLPFC gray matter with the phenol-chloroform method, and bisulfite conversion of DNA was performed with the EZ DNA methylation kit (Zymo Research, Irvine, CA, USA) for analysis of DNAm using the HM450K array.

Postmortem brains from which they generated these data were recovered during routine autopsies at the Offices of the Chief Medical Examiners of the District of Columbia and of the Commonwealth of Virginia, Northern District following informed consent from legal next-of-kin for donation to the National Institute of Mental Health Brain Tissue Collection at the National Institutes of Health in Bethesda, Maryland. Details of the postmortem brain donation process have been previously described (Deep-Soboslay et al., 2005; Jaffe et al., 2016; Lipska et al., 2006). Briefly, consensus Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (**DSM-IV**) diagnoses were made by psychiatrists based on clinical information obtained via structured interviews with the next-of-kin, including the Structured Clinical Interview for DSM-IV–clinician version (First et al., 1997) and the NIMH psychological autopsy interview, and psychiatric record reviews with the Diagnostic Evaluation After Death (Zalcman et al., 1983). All brains underwent neuropathological examination and those with evidence of neurological disorder were excluded. SZ group subjects met DSM-IV criteria for schizophrenia or schizoaffective disorder, and NPC group subjects did not meet DSM-IV criteria for any psychiatric or substance-related disorders and toxicology screening at time of death excluded acute drug and/or alcohol use.

For the present study, data from 232 NPC subjects (160 males, 72 females) and 195 SZ subjects (119 males, 76 females), all with ages >17 years of age, were downloaded from Gene Expression Omnibus (**GEO**) (GSE74193). Ages ranged from 17 to 85 years of age for NPC subjects and 17–97 years of age for SZ subjects. Additional phenotypic information for the subjects in this dataset, including antipsychotic use at time of death, illness duration, and manner of death, was obtained from the authors of the original study (Table 1).

2.1.2. Blood datasets

2.1.2.1. Blood dataset #1. These data were generated by Hannon and colleagues (Hannon et al., 2016) using whole blood DNA of subjects from The University College London (UCL) case–control sample (Datta et al., 2010). They extracted DNA from frozen whole blood samples with the phenol-chloroform method, and bisulfite conversion of DNA was performed with the EZ-96 DNA methylation kit (Zymo Research, Irvine, CA, USA) for analysis of DNAm using the HM450K array.

Subjects in the UCL case-control sample were recruited from UK National Health Service (**NHS**) clinics in London and South England. Subjects were included only if both parents were of English, Irish, Welsh, or Scottish descent and if three out of four grandparents were of the same descent. SZ group subjects were recruited based on having a clinical diagnosis of SZ and then interviewed using the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (**SADS-L**) (Spitzer and Endicott, 1977), those that received a probable diagnosis of SZ per Research Diagnostic Criteria (**RDC**) were included. Individuals with bipolar disorder, schizoaffective disorder bipolar type, and SZ associated with brain damage were excluded. NPC group subjects interviewed with the SADS-L screening questions, those with neither a personal history of an RDC-defined psychiatric disorder nor a family history of SZ, bipolar disorder, or alcohol use disorder were included (Datta et al., 2010).

For the present study, data from 322 NPC and 353 SZ subjects were downloaded from GEO (GSE80417). Chronological age information was missing for 17 NPC and 20 SZ subjects, and there was an evident error in the entry of chronological age for one subject from each of the NPC and SZ groups (recorded as 891 and 883 years of age, respectively). Analysis was performed on the 304 NPC subjects (135 males, 169 females) and 332 SZ subjects (242 males, 90 females) with correct chronological age information (Table 1). Ages ranged from 18 to 87 years of age for NPC subjects, and 19–90 years of age for SZ subjects.

2.1.2.2. Blood dataset #2. These data were generated by Hannon and colleagues (Hannon et al., 2016) using whole blood DNA of subjects from

Table 1
Cohort characteristics.
Data for continuous variables are presented as group average ± SEM. NPC, non-psychiatric control; SZ, schizophrenia; M, male; F, female; W, white; B, black; ATOD, at time of death; AP+, antipsychotic medication positive; AP−, antipsychotic medication negative; NA, not available; Sm, smoker; NS, non-smoker; N, natural death; A, accidental death; H, homicide; Su, suicide; PMI, postmortem interval.

Cohort	Brain		Blood #1		Blood #2	
Group	NPC	SZ	NPC	SZ	NPC	SZ
Number	232	195	304	332	405	260
Sex	160 M, 72 F	119 M, 76 F	135 M, 169 F	242 M, 90 F	303 M, 102 F	177 M, 83 F
Race	100 W, 132 B	108 W, 87 B	304 W	332 W	405 W	260 W
Antipsychotics ATOD	0 AP+, 150 AP−, 82 NA	125 AP+, 67 AP−, 3 NA	–	–	–	–
Smoking cigarettes ATOD	54 Sm, 171 NS, 7 NA	131 Sm, 53 NS, 11 NA	–	–	–	–
Manner of death	179 N, 20 A, 31 H, 2 NA	128 N, 24 A, 36 Su, 7 NA	–	–	–	–
PMI (hours)	30.4 ± 0.97	39.1 ± 1.73	–	–	–	–

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