RESEARCH ARTICLE

ALCOHOL
CLINICAL & EXPERIMENTAL RESEARCH

Biological aging markers in blood and brain tissue indicate age acceleration in alcohol use disorder

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Abstract

Background: Alcohol use disorder (AUD) is associated with increased mortality and morbidity risk. A reason for this could be accelerated biological aging, which is strongly influenced by disease processes such as inflammation. As recent studies of AUD show changes in DNA methylation and gene expression in neuroinflammation-related pathways in the brain, biological aging represents a potentially important construct for understanding the adverse effects of substance use disorders. Epigenetic clocks have shown accelerated aging in blood samples from individuals with AUD. However, no systematic evaluation of biological age measures in AUD across different tissues and brain regions has been undertaken.

Methods: As markers of biological aging (BioAge markers), we assessed Levine's and Horvath's epigenetic clocks, DNA methylation telomere length (DNAmTL), telomere length (TL), and mitochondrial DNA copy number (mtDNAcn) in postmortem brain samples from Brodmann Area 9 (BA9), caudate nucleus, and ventral striatum ($N=63–94$), and in whole blood samples ($N=179$) of individuals with and without AUD. To evaluate the association between AUD status and BioAge markers, we performed linear regression analyses while adjusting for covariates.

Results: The majority of BioAge markers were significantly associated with chronological age in all samples. Levine's epigenetic clock and DNAmTL were indicative of accelerated biological aging in AUD in BA9 and whole blood samples, while Horvath's showed the opposite effect in BA9. No significant association of AUD with TL and mtDNAcn was detected. Measured TL and DNAmTL showed only small correlations in blood and none in brain.

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Conclusions: The present study is the first to simultaneously investigate epigenetic clocks, telomere length, and mtDNAcn in postmortem brain and whole blood samples in individuals with AUD. We found evidence for accelerated biological aging in AUD in blood and brain, as measured by Levine's epigenetic clock, and DNAmTL. Additional studies of different tissues from the same individuals are needed to draw valid conclusions about the congruence of biological aging in blood and brain.

KEYWORDS
alcohol use disorder, biological aging, copy numbers of mitochondrial DNA, epigenetic clocks, telomere length

INTRODUCTION

Excessive alcohol use is a major risk factor for all-cause mortality, premature death, and increased neurodegenerative disease susceptibility (World Health Organization, 2018). In individuals with alcohol use disorder (AUD), age-related morbidities are especially prevalent (Stephenson et al., 2021; Topart et al., 2020). It has been found that individuals with AUD have a reduced life expectancy compared with nonaffected individuals (Bøstrand et al., 2022; Kresovich et al., 2021); specifically, a population-based study reported that persons with AUD had a decreased life expectancy of 24–28 years when compared to the general population in several Scandinavian countries (Westman et al., 2015). We posit that the increased mortality in AUD could in part be explained by accelerated biological aging.

Biological aging describes the progressive deterioration of biological functions, in contrast to chronological aging, which represents the time since birth (Horvath, 2013). Molecular biomarkers to estimate biological aging (BioAge markers) include epigenetic clocks, telomere length (TL), and copy numbers of mitochondrial DNA (mtDNAcn; Horvath & Raj, 2018; Longchamps et al., 2020; Lu et al., 2019).

Telomeres consist of short tandem DNA repeats and specific proteins at the end of eukaryotic chromosomes; they provide chromosomal integrity and their length naturally declines during chronological aging (Blackburn, 2005; Vaiserman & Krasnienkov, 2021). In addition, TL is influenced by lifestyle factors and a shortening of TL is associated with several disease phenotypes (Breitling et al., 2016; Marioni et al., 2015). While there are inconclusive results regarding the influence of alcohol consumption on TL, individuals with AUD typically show shortened TL compared with healthy controls (Navarro-Mateu et al., 2021). TLs are most commonly measured by quantitative real-time PCR or fluorescence in situ hybridization coupled with flow cytometry (Gutierrez-Rodrigues et al., 2014; Lai et al., 2018). However, an epigenetic estimate for telomere length (DNAmTL) in leukocytes was also developed which was shown to outperform PCR-measured TL in the prediction of mortality and disease-related events (Lu et al., 2019). So far, no study evaluated telomere length in brain tissue in the context of AUD.

Epigenetic clocks are biomarkers of aging based on DNA methylation (DNAm; Horvath & Raj, 2018). There are several epigenetic clocks available, the most prominent being Horvath's epigenetic clock, which was trained on a variety of different tissues and typically shows a high correlation with chronological age (Horvath & Raj, 2018). More recent epigenetic clocks such as Levine's PhenoAge provide estimates of biological age by including the effects of disease processes on epigenetic age into the regression model. For instance, Levine's clock was trained on different blood markers for inflammation, immune, liver, kidney, and metabolic functioning (Levine et al., 2018). Epigenetic age acceleration (ageAccel) refers to the residuals when epigenetic age is regressed on chronological age, a positive residual is indicative of accelerated biological age. Similar to TL, epigenetic age and ageAccel are influenced by several environmental factors such as diet, physical activity, weight, stress, and also alcohol intake (Horvath et al., 2014; Pal & Tyler, 2016). An ageAccel of 2.22 years was seen in peripheral blood of patients with AUD compared with healthy controls (Luo et al., 2020). Furthermore, a recent study found an increased PhenoAge and decreased DNAmTL in postmortem human brain samples in different substance use disorders (SUDs) when compared to control samples (Cabrera-Mendoza et al., 2023). It has also been shown that the age acceleration in AUD can be partly reversed with abstinence (Zindler et al., 2022). These findings suggest accelerated biological aging in blood and brain tissue in individuals with AUD.

Another biomarker of biological aging is mtDNAcn, representing the mtDNA level in a cell which is closely related to mitochondrial enzyme activity and ATP production (Jeng et al., 2008). Reductions in mtDNAcn are associated with cumulative dysfunction of the mitochondria and a variety of diseases, such as cardiovascular (Ashar et al., 2017) and neuropsychiatric (Fries et al., 2017) diseases. At the same time, mtDNAcn is an important marker for inflammation and shows a general decrease with chronological age (Mengel-From et al., 2014). mtDNAcn can be measured by quantitative real-time PCR, by genotyping using microarrays, or whole genome sequencing (Longchamps et al., 2020). The first evidence of an association with AUD comes from a recent population-based longitudinal study in Swedish women demonstrating a negative association of mtDNAcn with AUD in peripheral blood (Wang et al., 2022) but it remains unclear whether mtDNAcn is also associated with AUD in the brain.

While studies in peripheral blood have shown accelerated biological aging in AUD, little is known about the extent of this effect in postmortem brain tissues. A study in the prefrontal cortex reported a trend toward negative ageAccel in the brain of individuals with AUD when using Horvath's clock to calculate epigenetic age (Rosen et al., 2018). In contrast, the more recent study by Cabrera-Mendoza et al. suggests positive ageAccel in the prefrontal cortex in AUD with estimates based on the PhenoAge epigenetic clock. The prefrontal cortex and particularly BA9 are of high interest in addiction research, because of their role in executive control (Goldstein & Volkow, 2011). For the present study, we additionally focused on two other brain regions that are part of the neurocircuitry of addiction: the ventral striatum and the caudate nucleus. The ventral striatum is composed of the olfactory tubercle and the nucleus accumbens (NAcc) and plays a central role in reward processing. Dopaminergic neurons from the ventral tegmental area project into the NAcc via the mesolimbic pathway, which is thought to play a role in the rewarding effects of addictive drugs (Taber et al., 2012; Volkow & Morales, 2015). The NAcc is particularly crucial in incentive learning and in the development of AUD where a recalibration of the dopamine-activation/reward threshold is hypothesized (Cui et al., 2013). The caudate nucleus is part of the dorsal striatum. In the development of AUD, it is thought that a shift occurs from goal-directed drinking behavior to habit formation and activation in the caudate nucleus is associated with reinforcement learning essential for habit formation (Galandra et al., 2018). Studying biological aging not only in blood but also the human brain is of particular importance as DNAm profiles, TL, and mtDNAcn are all highly tissue-specific (Demanelis et al., 2020; D'Erchia et al., 2015; Lokk et al., 2014). Also, TL declines with age at different rates depending on the tissue with a more rapid decline in blood compared with the brain (Demanelis et al., 2020). Furthermore, SUDs are primarily considered as brain disorders and different brain regions have been associated with the development and maintenance of addiction phenotypes (Volkow et al., 2016), altogether highlighting the importance of investigating biological aging markers directly in the brain. In a recent study, we identified DNAm and gene expression differences in the ventral striatum (VS) and caudate nucleus (CauN) between cases with severe AUD and individuals without AUD using a brain region-specific approach. In particular, we detected enrichment for inflammation and immune response processes, which are themselves associated with biological aging (Zillich, Poisel, Frank, et al., 2022).

The previously observed consistently positive association between ageAccel and AUD in whole blood suggests that biological aging is accelerated in AUD and we hypothesize that this effect can be observed in both peripheral blood and postmortem human brain tissue. Here, we examined biological aging by assessing TL, epigenetic clocks, and mtDNAcn in a cross-tissue approach with a multi-method paradigm. Using this approach, our study is the first that simultaneously assessed TL, epigenetic clocks, and mtDNAcn from postmortem brain and whole blood samples in individuals with AUD and compared them to nonaffected individuals to address the hypothesis of accelerated biological aging in AUD.

MATERIALS AND METHODS

Samples

A total of 281 postmortem human brain samples and 179 blood samples were analyzed in the present study. An overview of the different samples, sample characteristics, such as age, sex and postmortem interval (PMI), descriptive statistics, and the generated markers of biological aging is found in Table 1. We tested differences in demographic variables and covariates between individuals with and without AUD using t-tests for continuous and chi-squared tests for categorical variables.

Postmortem human brain samples

Postmortem human brain tissue samples from three different brain regions were obtained from the New South Wales Brain Tissue Resource Centre at the University of Sydney, Australia (NSWBTRC). Brain regions of interest were BA9 ($N=91$), CauN ($N=94$), and VS ($N=63$). Individuals with AUD met DSM-IV criteria of alcohol dependence (as assessed by a retrospective assessment of medical records by an experience mental health clinician) and consumed at least 80 g of alcohol daily. Detailed descriptions of the sample, methods for DNA extraction, the generation of methylation data, and estimation of cell type proportions can be found in Zillich, Frank, Streit, et al. (2022).

An additional 16 samples originated from The University of Texas Health Science Center at Houston (UTHealth) Brain Collection, which is a collaboration with the Harris County Institute of Forensic Science. This sample has been described in Zillich, Poisel, Streit, et al. (2022).

Another subset of brain samples ($N=17$) was obtained from the Tampere Sudden Death Study cohort (TSDS). These samples are a subset of a cohort collected during forensic autopsies and comprise individuals who died in hospitals of the Pirkanmaa Hospital District area (Finland) during 2009–2015. All sample donors ($N=17$), in this current study, met the following inclusion criteria based on autopsy reports and medical records: age > 18, no history of neurodevelopmental, severe psychiatric, or SUDs, except for AUD and tobacco use disorder. These brain samples have been described in detail in Kärkkäinen et al. (2021).

Whole blood samples

A clinical sample of male patients who underwent alcohol withdrawal treatment and healthy male controls was analyzed. Whole blood samples were obtained at the Central Institute of Mental Health (CIMH) Mannheim, Germany, and the addiction ward of the Ludwig Maximilian University (LMU) hospital for psychiatry and psychotherapy in Munich, Germany. Inclusion criteria were alcohol dependence diagnosed according to DSM-IV criteria and

TABLE 1 Descriptive statistics and sample overview.

Tissue	NSWBTRC			TSDS	UTHealth		CIMH and LMU
	BA9	CauN	VS	BA9	BA9	Whole blood	Whole blood
N Total	91	94	63	17	16	16	179
AUD	39	44	30	9	8	6	95
Control	44	50	33	8	8	10	84
Age	56.8 (10.4)	56.9 (10.3)	56.2 (10.7)	63.2 (12.3)	51.1 (13.2)	48.8 (15)	47.4 (8.8)
Sex (%male)	59.90%	63.80%	74.60%	58.80%	87.50%	100.00%	100.00%
Smokers (%)	50%**	61.7%**	63.5%*	53%	50%	50.0%	48.6%**
Ethnicity	EA 100%	EA 100%	EA 100%	FA 100%	EA 62.5%, AA 18.75%, other 18.75%	EA 68.75%, AA 12.5%, other 18.75%	EA 100%
PMI (hours)	34.03 (16.6)	32.80 (16.4)	33.22 (17.0)	120 (40.7)	30.93 (9.1)	29.00 (7.7)	—
N TL	78	94	63	—	—	—	179
N mtDNAcn	78	94	63	—	—	—	179
N ECs	91	94	63	17	16	16	179

Note: AUD and control comparison: *$p<0.05$, **$p<0.001$.

Abbreviations: —, not measured; AA, African Ancestry; AUD, alcohol use disorder; BA9, Brodmann Area 9; CauN, caudate nucleus; CIMH, Central Institute of Mental Health; EA, European Ancestry; EC, epigenetic clocks; FA, Finnish Ancestry; LMU, Ludwig Maximillian University; mtDNAcn, mitochondrial DNA copy numbers; NSWBTRC, New South Wales Brain Tissue Resource Centre; TL, telomere length; TSDS, Tampere Sudden Death Study; UTHealth, The University of Texas Health Science Center at Houston; VS, ventral striatum.
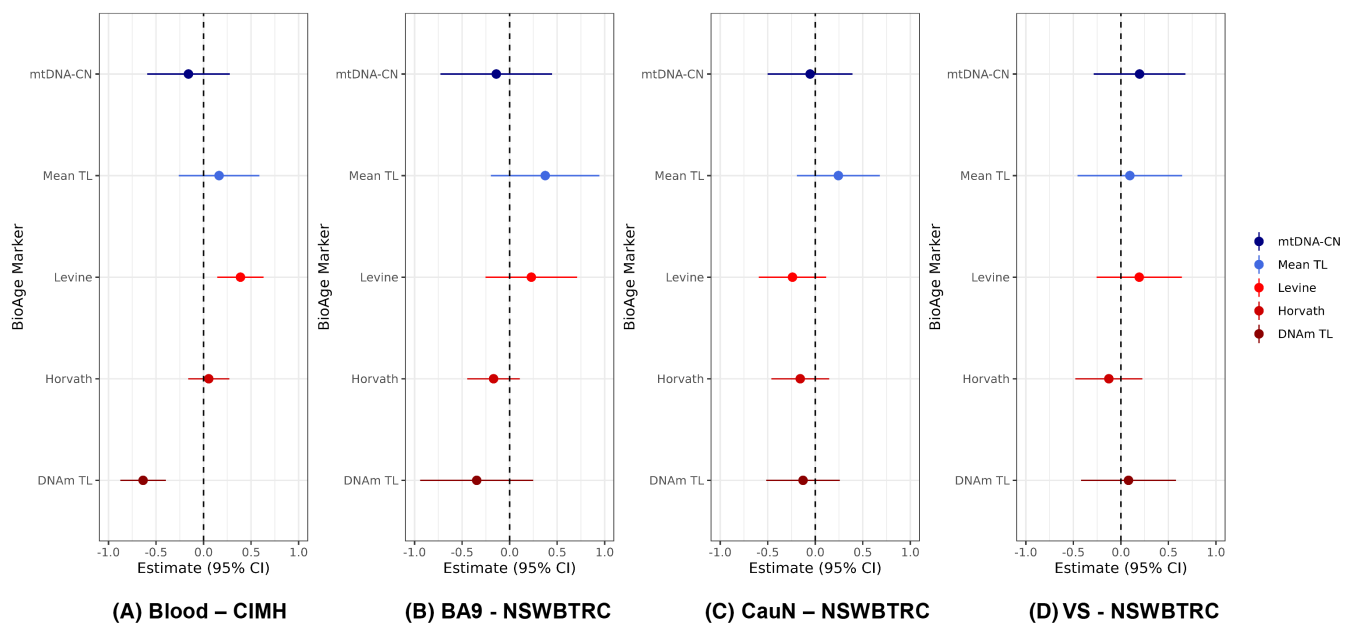

FIGURE 1 Epigenetic age estimates in AUD based on linear regression models for different BioAge markers in (A) whole blood, (B) Brodmann Area 9 (BA9; part of the prefrontal cortex), (C) caudate nucleus (CauN), and (D) ventral striatum (VS) while adjusting for covariates. Error bars represent the confidence interval (CI). CIMH cohort: $N=179$ blood samples, NSWBTRC cohort: $N=91$ BA9 samples, $N=94$ CauN samples, and $N=63$ VS samples.

patients had to present with an alcohol withdrawal syndrome, defined as a CIWA-Ar score above four in the first 48 h after admission. A detailed description of the cohort can be found in Witt et al. (2020).

For a subsample of the UTHealth cohort, DNA methylation levels from whole blood samples were also available ($N=12$).

DNA methylation

DNA was extracted, DNA methylation levels determined, and raw data preprocessed as described in Zillich, Frank, Streit, et al. (2022). In brief, the Illumina Infinium EPIC BeadChip v1.0 (Illumina, San Diego, CA, USA) was used for DNA methylation measurement. All

samples were randomized for age, sex (if applicable), and AUD status on processing plates and BeadChips. We removed samples if they showed a missing rate >0.1 and probes if the call rate was <0.95. We also tested whether methylation- and phenotype-based sex information overlapped, which was the case for all samples. Obtained intensity data were quantile-normalized and converted to methylation values (beta values) using an adapted version of the approach proposed by Lehne et al. (2015).

Measures of biological aging

Telomere length

Quantitative PCR (qPCR) was performed in triplicates in a reaction volume of 10 μL containing 5 ng DNA, 5 μL iTaq Universal SYBR Green Supermix (Bio-Rad, USA), 200 nM telomere primers, and 200 nM single copy gene primers as initially described by Cawthon (2002). Beta-Globin was used as single copy gene as described by O'Callaghan and Fenech (2011). Primer sequences are listed in Table S1. Reactions were performed on a CFX384 Real-Time Cycler (Bio-Rad, USA), with the same PCR settings for both targets (qPCR settings: 95°C 3 min, 35× (94°C 15 s, 54°C 1 min), melt curve 65°C to 95°C, increment 0.5°C, 5 s). Data from multiple qPCR runs were calibrated and normalized using CFX Maestro 2.3 software (Bio-Rad, USA). TLs were calculated using the ΔΔCT method by comparing cycle threshold (CT) values of the samples to the internal laboratory cell lines with known TLs: HEK293 (human embryonic kidney 293 e), THP-1 (human monocytic), and U251 (human glioblastoma).

Mitochondrial DNA copy numbers

mtDNAcn was quantified on a CFX384 Real-Time Cycler (Bio-Rad, Hercules, USA), following a protocol described by Phillips et al. (2014). Primer sequences are reported in the supplementary material (Table S2). Multiplex-PCRs were performed in triplicates in a 10 μL final volume containing 5 ng human genomic DNA, 5 μL SsoAdvanced Universal Probe Supermix (Bio-Rad, USA), and primers and probes as indicated in Table S2. Cycling conditions on a CFX384 Cycler (Bio-Rad, USA) were 94°C for 2 min followed by 35 cycles of 94°C for 10 s and 55°C for 1 min. Inter-run calibration and data normalization were performed using CFX Maestro 2.3 Software (Bio-Rad, USA). mtDNAcn was quantified using the ΔΔCT method compared with known mtDNAcn of the internal laboratory cell lines HEK293, THP-1, and U251.

Epigenetic clocks

Epigenetic clocks were calculated using the R package methylclock v1.4.0 (Pelegí-Sisó et al., 2021), based on quantile-normalized beta values, preprocessed as described above and in Zillich, Frank, Streit, et al. (2022). We computed Horvath's (2013) epigenetic clock to investigate chronological age as a negative control and Levine's (Levine et al., 2018) epigenetic clocks to operationalize biological aging, as well as an epigenetic clock for telomere length (DNAmTL; Lu et al., 2019). In methylclock, a defined set of respective clock CpG sites is extracted from the methylation data matrix and epigenetic clocks and epigenetic age acceleration are determined in the sample of interest using regression models. Of the 353 CpG sites in Horvath's epigenetic clocks, 19 (5.4%) were missing, and of the 140 CpG sites in the DNAmTL clock, four (2.9%) were missing after quality control. We did not observe missings in the 513 CpG sites in Levine's PhenoAge. In addition, methylclock has the advantage that data can be processed locally which improves data security.

Statistical analyses

All statistical analyses were performed using R version 4.2.1 (R Core Team, 2021). TL and mtDNAcn were winsorized to a maximum deviation from the mean of three standard deviations. All BioAge markers were standardized using the scale() function in R to ensure the comparability of effects. We calculated Pearson correlations to examine the relationship between chronological age and AUD with the BioAge markers and covariates, such as age, sex (if applicable), and smoking.

Next, we calculated linear models using the different BioAge markers as dependent and AUD status as independent variables. All models were adjusted to capture extrinsic epigenetic age acceleration and therefore included, in addition to self-reported smoking and sex (if applicable), age, and the estimated cell-type proportions as covariates. Cell-type proportions were estimated according to the Houseman method (Houseman et al., 2012) using the estimateCell-Counts() function of the minfi package v1.44.0 (Aryee et al., 2014) with the "Blood" reference data for the peripheral blood cohorts and the "DLPFC" reference for all postmortem brain samples. We observed group differences in cell type composition between cases and controls in the patient cohort and in BA9 (Figures S1A,B). To avoid multi-collinearity, we excluded the granulocyte cell type proportion showing the highest variance inflation factor. In cases where test p-values and 95% confidence interval of the effect estimate disagreed on significance of the respective finding, the latter was preferred.

Next, we performed a meta-analysis of the BA9 samples from the NSWBTRC, the TSDS, and the UTHealth cohorts based on the estimated marginal means and standard deviations using the meta-cont() function of R package meta v6.2.1 (Schwarzer, 2007).

We further explored the relationship between the DNAmTL epigenetic clock and TL measured by PCR using Pearson correlation analysis. In addition, we investigated the correlation between the epigenetic clocks in the UTHealth postmortem brain and blood samples using the Spearman correlation method instead of Pearson due to the small sample size (n = 12).

RESULTS

BioAge markers and chronological age

Across all subsets, the majority of BioAge markers showed small to medium statistically significant correlations with chronological age. Shorter TL and shorter DNAmTL were indicative of older age (TL: $-0.35 \geq r \geq -0.10$, DNAmTL: $-0.64 \geq r \geq -0.40$) in all cohorts and Levine's and Horvath's epigenetic clocks showed strong positive correlations with chronological age (Levine: $0.51 \geq r \geq 0.76$, Horvath: $0.70 \geq r \geq 0.86$). No significant association between mtDNAcn and age was detected (all $p \geq 0.26$). Correlation plots for all samples are shown in Figure S2.

BioAge markers and AUD status

In the CIMH/LMU sample, AUD was positively associated with Levine's epigenetic clock, pointing toward an increased biological age in individuals with AUD ($b = 0.39$, $p = 0.002$). In addition, DNAmTL was negatively associated with AUD status ($b = -0.64$, $p < 0.001$), see also Figure 1. For Horvath's epigenetic clock, mtDNAcn, and TL, no significant difference was detected between individuals with and without AUD.

In the postmortem brain samples, all of the confidence intervals included zero, although a similar trend to the blood samples was observed in BA9. A summary of regression coefficients for all samples is provided in Table S3 and Figure 1.

Meta-analysis of epigenetic clocks in BA9

For BA9, DNA methylation data from two additional cohorts were available, which we then meta-analyzed resulting in a total sample size of $N = 124$. Here, we observed effects consistent with the findings in whole blood: Individuals with AUD showed an increase in epigenetic age as measured by Levine's clock ($MD = 0.25$, $p < 0.0001$; Figure 2A). In contrast, Horvath's clock indicated a negative age acceleration in AUD ($MD = -0.18$, $p < 0.0001$; Figure 2B). DNAmTL was reduced in individuals with AUD compared with controls ($MD = -0.35$, $p < 0.0001$; Figure 2C).

Exploratory analyses

DNAmTL and qPCR-measured TL

The simultaneous assessment of qPCR-measured TL and DNAmTL allows for a direct comparison between the two estimates, which both correspond to TL as the assessed biological feature. No significant association was detected between TL and DNAmTL in BA9 ($r = -0.04$, $p = 0.71$), in CauN ($r = 0.11$, $p = 0.28$), or in VS ($r = 0.04$, $p = 0.78$). At the same time, there was a medium and statistically significant correlation between the two measures in blood ($r = 0.28$, $p < 0.001$; CIMH/LMU).

Consistency of epigenetic clocks in blood and brain

In the UTHealth cohort, DNAm data were available for both post-mortem brain and blood samples. Therefore, we tested the correlation of the epigenetic clocks in the different tissues. Overall, there was a positive correlation of the epigenetic clocks between postmortem blood and brain samples, as signified by high Spearman correlation coefficients. Horvath's clock showed a strong overlap ($rho = 0.81$, $p = 0.002$), while the correlations for Levine's clock ($rho = 0.72$, $p = 0.01$) and DNAmTL ($rho = 0.63$, $p = 0.03$) were smaller. Scatterplots are displayed in Figure S3.

DISCUSSION

In the present study, we investigated the hypothesis of accelerated biological aging in AUD by examining different BioAge markers in postmortem human brain tissue and whole blood samples of individuals with and without AUD.

We found that most BioAge markers were associated with chronological age across the entire sample. Testing this is of particular importance in postmortem human brain tissue as little is known about the effects of degradation processes on the performance of BioAge markers.

Interestingly, the largest estimates for AUD were observed for epigenetic clocks. In line with the hypothesis of accelerated biological aging in AUD, we observed age acceleration for Levine's PhenoAge both in BA9 and in whole blood samples. PhenoAge is supposed to capture CpG sites that change methylation levels not only with age, but also due to environmental and disease processes (Levine et al., 2018). Accelerated PhenoAge is known to be associated with pro-inflammatory and interferon pathways (Han et al., 2022; Levine et al., 2018). Our findings from the present study might therefore point toward increased inflammation in the brains of individuals with AUD, which is in line with our previous findings in these cohorts (Witt et al., 2020; Zillich, Frank, Streit, et al., 2022).

In contrast to PhenoAge, Horvath's epigenetic clock is more reflective of chronological age and CpG sites were not selected to reflect disease markers (Horvath, 2013). Therefore, large group differences would not be expected for this epigenetic clock. Nevertheless, we observed a negative estimate for age acceleration in BA9 using Horvath's clock. Rosen et al. (2018) observed a similar result and the authors suggested that a possible explanation could be differences in AUD severity. The majority of the sample was diagnosed with alcohol abuse. While there might be sample overlap with the Rosen study in the NSWBTRC samples, the TSDS cohort consisted of cases with severe AUD and the negative age acceleration was especially pronounced in this subsample. Therefore, this explanation seems unlikely for our study. Another explanation could be altered methylation levels of CpG sites in Horvath's clock that do not provide equally valid estimates in postmortem human brain tissue compared with those in blood. This assumption is supported by the estimated marginal means in the control samples that indicated an age acceleration. Furthermore, it is
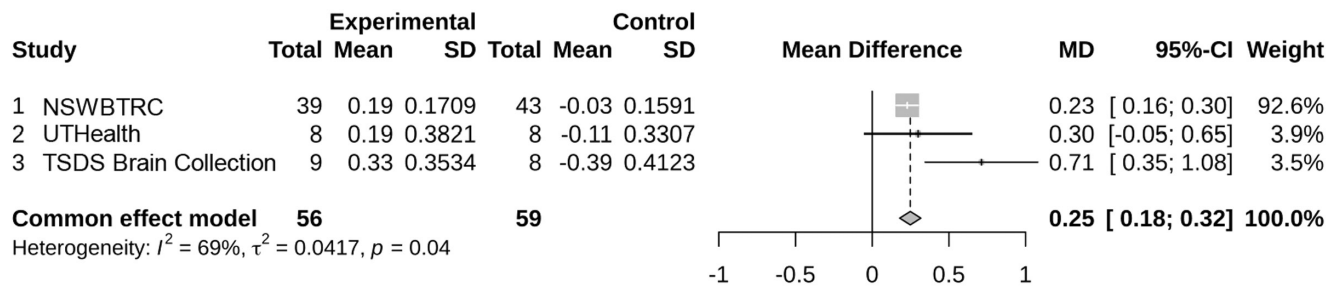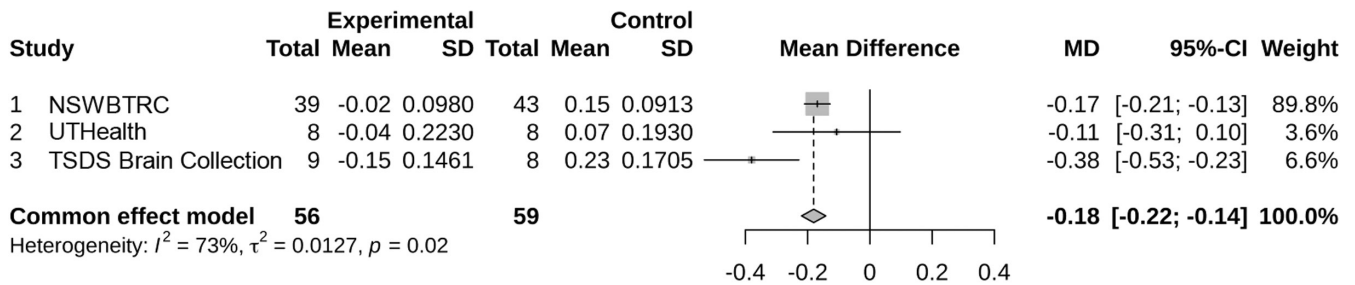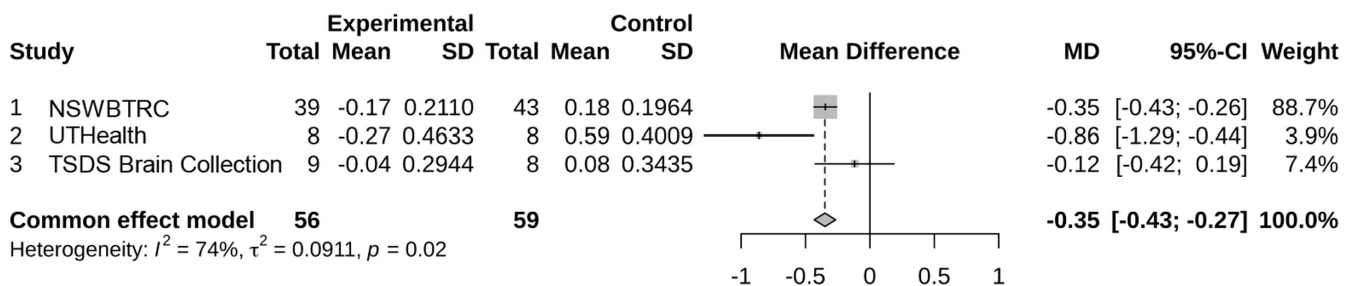
FIGURE 2 Results of the meta-analysis on epigenetic age acceleration in $N = 124$ BA9 postmortem human brain tissue samples, for the BioAge markers (A) Levine's and (B) Horvath's epigenetic clock, and (C) DNA methylation telomere length (DNAmTL). Error bars represent standard deviation. Evaluated cohorts were based on $N = 91$ (NSWBTRC), $N = 16$ (UTHealth), and $N = 17$ (TSDS Brain Collection) individuals.

possible that additional variables for which no adjustment was possible such as cause of death or medication prior to death might contribute to the observed negative estimate.

In the single cohort analyses of postmortem human brain tissue, none of the BioAge markers showed a statistically significant association with AUD. While we did observe significant p-values, all confidence intervals included zero, which is why the direction of the effect should not be interpreted. A likely explanation for this is the smaller sample size of the postmortem brain cohorts in combination with a smaller expected effect. While it is known that TL decreases with age, this effect is less pronounced in the brain tissue (Demanelis et al., 2020) because most cells do not proliferate. In our sample, there was a small correlation between age and TL in all brain regions and it is likely that a possible effect for AUD is too small to be detected in a sample size like ours.

One consideration that emerges from this study is the lack of association between DNAmTL and TL as measured by PCR. For BA9, this could be explained by the fact that DNAmTL was not trained on postmortem brain DNA methylation data (Lu et al., 2019), and in contrast to PhenoAge, it was also not validated for this type of tissue. DNAmTL was highly correlated in brain and blood samples of the same individuals, although this finding should be interpreted with caution, due to the small sample size. However, in blood samples one would expect a high correlation between the estimates. In recent studies, DNAmTL has been shown to have predictive value for a variety of disease phenotypes, such as HIV (Breen et al., 2022) and schizophrenia (Segura et al., 2022). The results from our study contribute to a growing body of evidence showing that DNAmTL and TL show little agreement and seem to differ in their way of assessing this biological feature (Hastings et al., 2022; Jung et al., 2023; Pearce et al., 2021).

It has to be noted that we observed heterogeneity between the cohorts in the meta-analysis. This could be explained by the different genetic backgrounds. Also, while we adjusted for all available covariates known to influence the BioAge markers, our AUD phenotypes did not include information about alcohol consumption or abstinence in the weeks prior to death or admission. More importantly, information about the age at onset of AUD was not available. Conceptually, we would expect an interaction between age at onset (relative to chronological age) and the BioAge markers, because the longer the disease is manifested, the more biological aging could progress. Further arise from postmortem human brain tissue, such as an unknown influence of cause of death on epigenetic aging und a relatively small sample size.

CONCLUSION

The present study is the first to jointly analyze different BioAge markers in peripheral blood samples and postmortem human brain tissue. It provides additional evidence for accelerated biological aging in AUD as measured by Levine's PhenoAge. Although we observed strong effects for DNAmTL as well, this marker showed little congruence with TL measured by qPCR, which should be considered when selecting methods for further studies on biological aging. Follow-up studies with larger and diverse samples, ideally with blood and brain samples from the same individual, and detailed phenotypic information about alcohol intake, AUD-related symptoms, and age at onset are needed to provide more insight into the mechanisms of accelerated biological aging in AUD.

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CONFLICT OF INTEREST STATEMENT

All authors report no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.