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ORIGINAL INVESTIGATION

Effect of alcohol use disorder on cellular aging

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Abstract

Rationale Human telomeres consist of tandem repeats at chromosome ends which protect chromosomal DNA from degradation. Telomere shortening occurs as part of natural aging; however, life stressors, smoking, drug use, BMI, and psychiatric disorders could disrupt cell aging and affect telomere length (TL). In this context, studies have evaluated the effects of alcohol consumption on TL; however, results have been inconsistent, which may reflect diverse drinking cut-offs and categorizations.

Objectives To help clarify this, the present study addresses the association of TL with alcohol use disorder (AUD), drinking behaviors, lifetime stress, and chronological age.

Methods TL was quantified as the telomere to albumin ratio (T/S ratio) obtained from peripheral blood DNA using the quantitative PCR assay, from 260 participants with AUD and 449 non-dependent healthy controls (HC) from an existing National Institute on Alcohol Abuse and Alcoholism (NIAAA) database.

Results AUD participants showed shorter TL compared to HC with both, age, and AUD, as independent predictors as well as a significant AUD with age interaction effect on TL. TL was also associated with impulsiveness in AUD participants. We did not observe an association between TL and chronicity of alcohol use, alcohol doses ingested, or childhood trauma exposures in either AUD or HC, although very few HC reported a history of childhood trauma.

Conclusion Our results support previous findings of telomere shortening with chronic alcohol exposures and show both an effect of AUD on TL that is independent of age as well as a significant AUD by age interaction on TL. These findings are consistent with accelerated cellular aging in AUD.

Keywords Telomere · Stress · Alcohol · Genetics

Introduction

Telomeres are repeats of conserved TTAGGG sequences at chromosome's ends that protect the genome from degradation and interchromosomal fusion. Specifically, telomeres protect genomic DNA against double-strand breaks and consequently against DNA end-joining and recombination (Zhu et al. 2018). Telomeres are not fully replicated during every cell division and become progressively shorter over the lifespan (Watson 1972; Olovnikov 1973). This occurs due to the 'end replication problem' in which the replication of the lagging strand is discontinuous, and the DNA polymerase is incapable to replicate this strand, generating a gap at the end (Wellinger 2014). When telomere length (TL) reaches a critical length, the cellular defense mechanisms are activated and cells enter a state of senescence or apoptosis (Victorelli and Passos 2017). Telomere shortening occurs as a natural component of aging. However, in the last decades, research has suggested that

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childhood trauma (Ridout et al. 2018), drug exposures (e.g., alcohol and drugs of abuse) (Coimbra et al. 2017; Levandowski et al. 2016), high BMI (Gielen et al. 2018), and psychiatric disorders such as anxiety and depression (Verhoeven et al. 2014; Darrow et al. 2016) could accelerate telomere erosion, thus negatively impacting TL. However, the association of TL with stress disorders is confounded by exposure to chronic as well as acute stressors with persistent effects (Rom and Reznick 2015). Moreover, stress events are associated with age (e.g., humans undergo distinct types of stressors during different life-time periods (Ridout et al. 2018)), high BMI and obesity (i.e., higher uncontrolled eating) (Järvelä-Reijonen et al. 2016), ethnicity, education levels, and socioeconomic status (Farah 2017).

Alcohol is the most widely used recreational drug and its abuse negatively affects health. The global status report on alcohol and health 2018 (World Health Organization 2018) estimated that in 2016, more than 3 million people died from harmful use of alcohol. Heavy alcohol consumption has been associated with premature aging and accelerates the onset of age-related diseases such as cardiovascular diseases, hypertension, diabetes, and cancer (World Health Organization 2018), all of which are associated with shorter TL (Blackburn et al. 2015). For this reason, studies have assessed the effects of alcohol consumption on TL. A recent meta-analysis reported that results on alcohol consumption and TL are unclear (Li et al. 2018). First, Pavanello et al. (2011) reported that TL in alcohol abusers was nearly half that of social drinkers and that TL was negatively associated with number of drinks per day. On the other hand, in a gastric cancer case-control study, longer TL was reported in ever drinkers compared to never drinkers, for both the cancer and control groups (Liu et al. 2009). Last, no significant association between alcohol consumption and TL in healthy volunteers was observed by Latifovic et al. (2016).

To help clarify the connection between alcohol consumption and TL, the present study aims to address the association of TL with alcohol use disorder (AUD) and alcohol drinking behaviors (doses consumed, dependence scores, and years of exposure) as a function of age and early life stress exposures. We hypothesized that (1) age and AUD would be negatively and independently associated with TL; (2) drinking behavior (i.e., lifetime and recent drinking quantities, alcohol dependence score) would be negatively associated with TL; and (3) childhood trauma would be an independent predictor of short TL.

Methods

Participants

Two hundred and sixty ($n = 260$) participants with AUD (current DSM IV or five diagnoses as per Structured Clinical Interview for DSM IV (SCID) (American Psychiatric Association 2000) and 449 non-dependent healthy controls (HC) were pooled from an existing National Institute on Alcohol Abuse database. Table 1 summarizes demographics and clinical characteristics. Participants provided written informed consent to participate in the study, which was approved by the Institutional Review Board at the National Institutes of Health. Participants were tested between July 2010 and August 2017.

Clinical assessment and behavioral measures

Clinical characteristics of AUD and HC participants were assessed through a standardized clinical interview (SCID IV) (American Psychiatric Association 2000), which also included self-reported ethnicity, height, weight, body mass index (BMI: weight (kg)/height^2 (m^2)), smoking status, and past and present clinical history.

AUD diagnosis was based on SCID interview for DSM-IV and DSM-5. DSM criteria for AUD include questions related to compulsion, relapse, and withdrawal. Additionally, participants also completed the alcohol use disorders identification test (AUDIT) (Saunders et al. 1993) that assesses alcohol consumption, drinking behaviors, and alcohol-related problems, and the Alcohol Dependence Scale (ADS) (Skinner and Allen 1982) that assesses severity of dependence based on psychological and physical symptoms of dependence. To quantify the amount of alcohol consumed, participants also completed the Timeline Followback (TLFB) to assess daily alcohol consumption in the 90 days prior to the study (Sobell and Sobell 1996) and the Lifetime Drinking History (LDH) to assess lifetime alcohol consumption (i.e., total LDH in grams, and heavy drinking years: 5 or more drinks/day on at least 5 different days per month) (Skinner and Sheu 1982). The Fagerstrom test was used as a measure of nicotine dependence (Heatherton et al. 1991).

Early life stress was assessed using the total score of childhood trauma questionnaire (CTQ) (Bernstein et al. 1994), which includes assessment of sexual, physical, and emotional abuse as well as physical and emotional neglect during early life and the early life stress questionnaire (ELS) (Sanders and Becker-Lausen 1995), which includes assessment of traumatic events during childhood, such as bullying and social rejection, life threatening illness, family conflict, and sexual abuse. The life event questionnaire (LEQ) was also applied to assess the negative and positive events that occurred in the past year, in the adult's life (Norbeck 1984).

Participants completed the Barrett Impulsiveness scale (BIS) (Patton et al 1995) and the Urgency, Premeditation (lack of), Perseverance (lack of), Sensation Seeking, Positive Urgency (UPPS-P) impulsive behavior scale (Whiteside and Lynam 2001) to measure impulsiveness, the Buss Perry Aggression questionnaire to measure aggression (Buss and

Table 1 Demographic and clinical characteristics of alcohol use disorder (AUD) and healthy control groups

Characteristics	AUD ($n = 260$)	Healthy controls ($n = 449$)	Statistics	p value
Age, years	44.06 ± ·73, $n = 260$	33.32 ± ·56 $n = 449$	$t = 11.52$ df = 707	$p < ·001$
BMI	27.82 ± ·34, $n = 258$	25.9 ± ·21, $n = 446$	$t = 4.98$ df = 702	$p < ·001$
Years of education	13.36 ± ·20, $n = 254$	15.78 ± ·15, $n = 445$	$t = 9.59$ df = 697	$p < ·001$
IQ score	81.38 ± 2.38, $n = 260$	105,5 ± 1,59, $n = 449$	$t = 8.70$ df = 707	$p < ·001$
Gender	F: $n = 73$, M: $n = 187$	F: $n = 201$, M: $n = 248$	$\chi^2 = 19.34$	$p < ·001$
Smoking status	C: $n = 137$; N: $n = 120$	C: $n = 39$; N: $n = 408$	$\chi^2 = 172.98$	$p < ·001$
Drinks per week	57.08 ± 2.90, $n = 249$	7.83 ± ·58, $n = 447$	$t = 21.36$ df = 694	$p < ·001$
Heavy drinking years	14.9 ± ·79, $n = 232$	1.51 ± ·23, $n = 401$	$t = 19.77$ df = 631	$p < ·001$
Total lifetime drink (g)	811,344 ± 55,218, $n = 232$	84,019 ± 9423, $n = 401$	$t = 16.62$ df = 631	$p < ·001$
AUDIT score	21.82 ± ·51, $n = 243$	5.31 ± ·22, $n = 440$	$t = 33.49$ df = 681	$p < ·001$
CTQ score	41.74 ± 1.03, $n = 255$	34.18 ± ·54, $n = 441$	$t = 7.07$ df = 694	$p < ·001$
ELS score	3.07 ± ·175, $n = 256$	1.78 ± ·10, $n = 443$	$t = 6.62$ df = 697	$p < ·001$
Negative events	23.43 ± 2.35, $n = 120$	9.315 ± ·81, $n = 197$	$t = 6.68$ df = 315	$p < ·001$
Positive events	2·03 ± 1.88, $n = 120$	2·6 ± 1.13, $n = 197$	$t = ·27$ df = 315	$p > ·05$

BMI body mass index; gender = (F) female and (M) male; smoking status = (C) current smoker and (N) non-smoker; CTQ score = Childhood trauma questionnaire; ELS score = early life events

Perry 1992), and the Brief Scale for Anxiety (BSA) and Montgomery-Asberg Depression Rating Scale (MADRS), both of which were calculated from the Comprehensive Psychopathology Rating Scale (CPRS) (Asberg et al. 1978) to measure anxiety and depression scores at baseline, respectively. The Wechsler Abbreviated Scale of Intelligence (WASI-II) subtests Matrix Reasoning and Vocabulary were used as a proxy for estimated general intelligence (Wechsler 1999).

Since ethnicity is an important variable when differences in TL are investigated due the cumulative burden of differential exposure to oxidative stress over the life course, we chose to use a panel of 2500 ancestry-informative markers and individual comparison to the 51 worldwide populations represented in the Human Genome Diversity Cell Line Panel of the Human Genome Diversity Project (HGDP) and Centre d'Etude du Polymorphisme Humain (CEPH), which includes 1051 individuals (http://www.cephb.fr/HGDP-CEPH-Panel), to characterize the ethnic origin of participants. Genotyping was performed using the Illumina human OmniExpressExome array (Illumina, San Diego, CA, USA) and compared to data from the Human HapMap 550 K array for the CEPH diversity panel. African and European Ancestry scores were calculated using Structure, version 2.2 (http://pritch.bsd.uchicago.edu/structure.html).

Telomere length assessment

Participants provided a whole blood sample for genomic testing. TL was assessed using monochrome multiplex quantitative PCR method, as described by Cawthon (2009). Firstly, genomic DNA was extracted from peripheral blood using QIAmp DNA blood kit (Qiagen, EUA) in accordance with the manufacturer's instructions, and DNA concentration set to 50 ng/μL. The standard curve was composed based on 6-points serial dilution, ranged from 800 to 25 ng. Reference DNA from a single person was used to establish a standard curve. Two standard curves, one for telomere and other for albumin gene, were estimated for each plate analyzed. To amplify telomere sequence, we used *Tel-g* 5′ ACACTAAG GTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT 3′ and *Tel-c* 5′TGTTAGGTATCCCTATCCCTATCCCTATCCCT ATCCCTAACA3′ primers. Albumin was used as single gene: *Alb-u* 5′CGGCGGCGGGCGGCGCGGGCTGGGCG GAAATGCTGCACAGAATCCTTG3′ and *Alb-d* 5′ GCCCGGCCCGCCGCGCCCGTCCCGCCGGAAAA GCATGGTCGCCTGTT3′. The efficiency reaction and specificity of the primer pairs as well as the absence of primer-dimers were examined prior to the experiment. PCR reaction was performed with a 384-well plate with 2 μL of DNA (100 ng/μL). One master mix was prepared containing: (1); 5 μL of SYBR® Select Master Mix (Life Technologies, USA); (2)·8 μL (900 ηM) of *Tel-g* primer; (3)·53 μL (600 ηM) of *Tel-c* primer; (4)·8 μL (900 ηM) of *Alb-d* primer; (5) 8 μL (900 ηM) of *Alb-u* primer; and (6) 07 μL of water. The final reaction volume was kept at 10 μL. Reactions were pipetted in triplicate for the standard curves and in duplicate for the other samples. In all reactions, a negative control without cDNA template (NTC) was tested.

PCR amplification was performed in three stages: (1) 15 min at 95 °C; (2) 2 cycles of 15 s at 94 °C, 15 s at 49 °C; and (3) 35 cycles of 15 s at 94 °C, 10 s at 68 °C, 19 s at 74 °C, 10 s at 85 °C and 19 s at 88 °C with signal acquisition at 74 °C and 88 °C. The 74 °C reads provided the Ct values for the amplification of the telomere template, and the 88 °C reads

provided the Ct values for the amplification of the albumin template. All reactions were performed with the ViiA™7 Real-Time PCR System (Life Technologies, USA). After thermal cycling and raw data collection, the standard curve was used to calculate the number of nanograms of standard DNA that matched the experimental sample for copy number of the telomere template (T) and for albumin (S). The ratio of (T/S) is proportional to the average TL.

Statistical analysis

A linear regression model was used to predict TL, with age and AUD as predictors. Table 2 provides zero-order Pearson's correlations between all variables, to explore for potential confounding variables. Since AUD was associated with age, gender, BMI, years of education, Fagerstrom score, and African ancestry, these variables were added as covariates in the model. Additionally, we also included the interaction of AUD and age in the regression model to assess the contribution of the interaction of both variables on TL. Supplementary Tables 1 and 2 provide zero-order Pearson's correlations between all variables for each group separately, including age, gender, BMI, Fagerstrom score, stress, ethnicity, and years of education to identify potential covariates. Additionally, to explore the variables related to stress exposures and TL, supplementary Table 3 provides a linear regression model with CTQ and ELS added as covariates in the model.

We used propensity score statistics to match AUD and HC groups for age, gender, BMI, years of education, Fagerstrom score, and African ancestry. The linear regression model for these matched samples was used to predict TL, with age and AUD as predictors (Supplementary Table 4).

To explore associations between drinking behavior and TL, we performed partial correlations for each group separately, for the following dependent variables: heavy drinking days and drinks per week (TLFB), heavy drinking years, and total lifetime drinking in grams (LDH), AUDIT, and ADS score. Additionally, we developed a score (drinking history), using structural equation modeling, to assess the "dose-response" association between ethanol consumption and TL, for AUD and HC separately, using the following variables: heavy drinking days, average drinks per day, drinks per week, heavy drinking years, total lifetime drinking history (LDH), and AUDIT. To explore the association between lifetime stress and TL, we performed partial correlations within each group separately with CTQ total score, ELS and negative events score (LEQ) as dependent variables. Additionally, a one-way ANOVA was performed to evaluate differences in TL for HC and AUD participants that experienced childhood abuse and/or neglect versus those who did not (Levandowski et al. 2016). The ELSE group consisted of participants who reported having been exposed to at least one moderate-to-severe type of child abuse or neglect according to the CTQ manual.

Exploratory partial correlations were performed for the following behavioral dependent variables: BIS (attention, motor, self-control, cognitive complexity, perseverance, cognitive instability, attentional impulsiveness, motor impulsiveness, and non-planning impulsiveness), Buss Perry aggression (physical aggression, verbal aggression, anger, and hostility measures), UPPS (urgency, premeditation, perseverance, sensation of seeking, and positive urgency), anxiety and depression baseline and IQ; correcting for age and years of education. To separate AUD effects, we also performed partial correlations with those variables within each group separately, also corrected for age and years of education.

Results

AUD and age predict TL

Table 1 summarizes the demographic and clinical characteristics of the samples. The AUD group was older (44 vs 33 years, $p < 001$), had higher BMI (27.8 vs 25.9 kg/m^2, $p < 001$) and fewer years of education (13.3 vs 15.7 years, $p < 001$), lower IQ (81.38 vs 105.5 IQ score, $p < 001$), compared to HC. As expected, the AUD group reported higher numbers of drinks per week (57.08 vs 7.09 drinks, $p < 001$), heavy drink years (14.9 vs 1.5 years, $p < 001$), and total life time drinking (811,344 vs 84,019 g, $p < 001$), compared with HC. Additionally, the AUD group had higher scores for AUDIT (21.32 vs 5.31, $p < 001$), higher CTQ total scores (41.74 vs 34.18, $p < 001$), higher early life stress on the ELS questionnaire (3.07 vs 1.08, $p < 001$), and experienced more negative events on the LEQ (23.43 vs 9.31, $p < 001$) than HC.

The zero-order correlations showed that AUD diagnosis ($r = -21$, $p < 001$) and age ($r = -23$, $p < 001$) correlated negatively with TL in both groups pooled together (Table 2). Moreover, the linear regression models showed that after correcting for age, gender, BMI, years of education, smoking status and African ancestry, both AUD ($\beta = -.424$, $p < 0001$, Fig. 1a) and age ($\beta = -.277$, $p < 0001$, Fig. 1b) predicted TL (Table 3), providing evidence for both AUD and age as independent predictors of TL. We also observe that the interaction between AUD and age had a significant effect on TL ($\beta = -.322$, $p = .002$). When the stress questionnaires (CTQ and ELS) were added as a covariate to the regression model (Supplementary Table 3), both AUD ($\beta = -.420$, $p < .005$), age ($\beta = -.255$, $p < .005$), and the interaction between AUD and age ($\beta = -.342$, $p < .005$) remained as independent predictors of TL. Neither CTQ ($\beta = .052$, $p > .005$) nor ELS ($\beta = -.039$, $p > .005$) were significant predictors of TL.

When AUD ($n = 127$) and HC ($n = 127$) groups were matched on age (39.91 ± 11.96 vs 39.72 ± 12.20 years), gender (93 males and 34 females vs 73 males and 54 females), BMI (27.68 ± 4.81 vs 27.37 ± 4.7 kg/m^2), years of education

Table 2 Zero-order correlations between telomere length (T/S) and potential covariates for both AUD and HC groups combined

	T/S ratio	Age	Sex	BMI	Edu	Aims Africa	Aims Euro	Fagerstrom score	AUD diagnosis	AUDIT	Drinks (week)	HD (years)	LDH (g)	CTQ	ELS	Negative events	Positive events	Total events
T/S ratio	1																	
Age	−.240**	1																
Sex	.069	−.058	1															
BMI	−.058	.241**	−.054	1														
Years of education	.088*	−.217**	.099**	−.164**	1													
African Ancestry	−.121**	.281**	−.009	.311**	−.390**	1												
European Ancestry	.077*	−.205**	−.039	−.252**	.304**	−.871**	1											
Fagerstrom score	−.093*	.253**	−.137**	.056	−.344**	.235**	−.175**	1										
AUD diagnosis	−.215**	.398**	−.165**	.185**	−.341**	.307**	−.237**	.437**	1									
AUDIT	−.077*	.314**	−.213**	.148**	−.325**	.203**	−.139**	.441**	.789**	1								
Drinks per week	−.112**	.269**	−.196**	.109**	−.301**	.234**	−.171**	.474**	.630**	.734**	1							
Heavy drinking years	−.133**	.509**	−.237**	.197**	−.317**	.297**	−.241**	.496**	.618**	.640**	.571**	1						
LDH (g)	−.145**	.433**	−.214**	.126**	−.317**	.257**	−.199**	.532**	.552**	.601**	.642**	.836**	1					
CTQ score	−.095*	.206**	.021	.231**	−.204**	.247**	−.219**	.265**	.259**	.265**	.240**	.198**	.201**	1				
ELS score	−.105**	.218**	.035	.164**	−.252**	.215**	−.171**	.193**	.243**	.250**	.172**	.186**	.159**	.674**	1			
Negative events	−.073	.085	−.073	.047	−.161**	.167**	−.121*	.264**	.352**	.386**	.248**	.239**	.235**	.340**	.369**	1		
Positive events	.117*	−.257**	−.094	.097	.026	.163**	−.186**	−.140*	−.015	−.076	−.096	−.023	−.052	.126*	.110*	.193**	1	
Total events	.023	−.102	−.107	.092	−.093	.214**	−.197**	.092	.228**	.214**	.109	.150*	.129*	.307**	.318**	.795**	.749**	1

T/S ratio telomere length; age in years; sex = female (1) versus male (0); *BMI* body mass index, *Edu* years of education; *Aims African* African ancestry informative marker, *Aims Euro* European ancestry informative marker, *AUD diagnosis* AUD (1) versus healthy control (0), *AUDIT* alcohol use disorders identification test; drinks per week in past 90 days (TLFB); *HD* heavy drinking years, *LDH* total lifetime drinking history (g), *CTQ* childhood trauma questionnaire, *ELS* early life events. Significance levels in bold: * $p < .05$, ** $p < .01$, *** $p < .001$

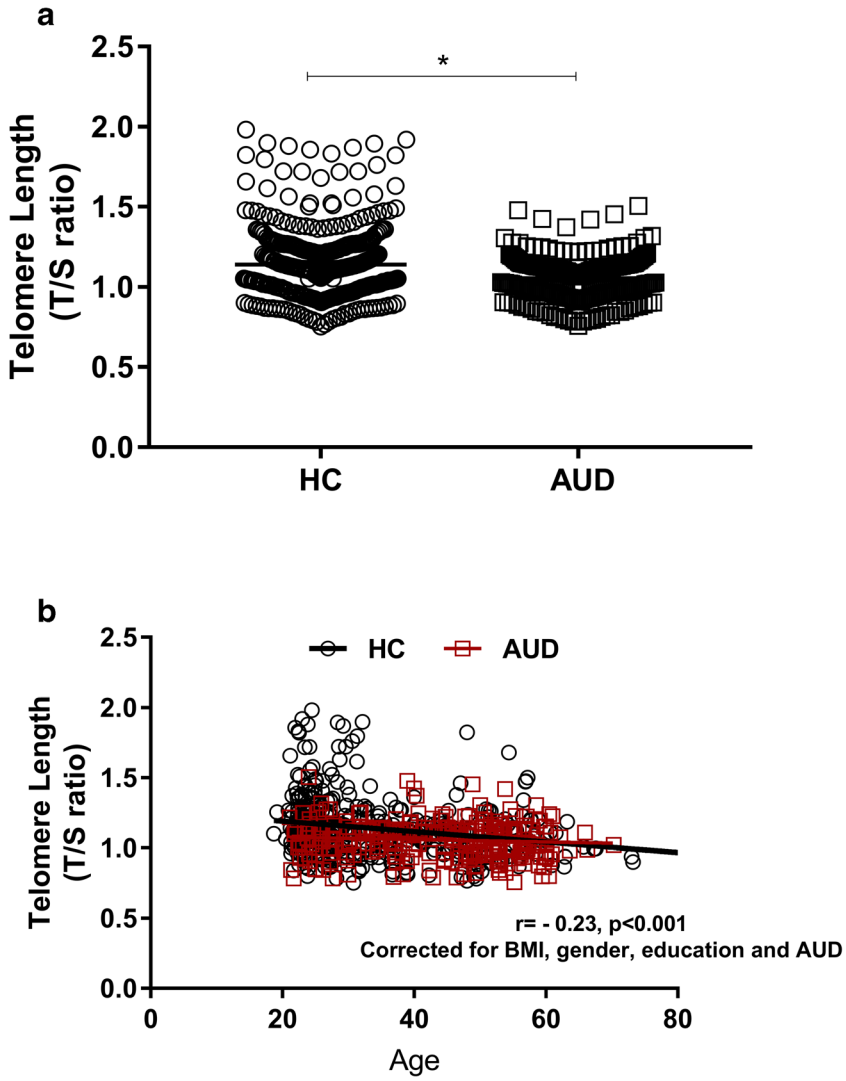

Fig. 1 **a** Comparison of telomere length of healthy controls (HC) and alcohol use disorder (AUD) groups. Horizontal lines indicate mean values. Mean and SE values: HC (1.141 ± ·009, n = 449) and AUD (1.056 ± ·008, n = 260). **b** Correlation between telomere length and age for AUD and HC groups, corrected for age, gender, BMI, years of education, and AUD

(14.23 ± 2.36 vs 15.54 ± 2.36 years), Fagerstrom score (0.67 ± 1.6 vs 0.57 ± 1.54 score) and African ancestry, both AUD ($\beta = -.716$, $p < .005$), age ($\beta = -.388$, $p < .005$) and the interaction between AUD and age ($\beta = -.594$, $p < .005$) remained independent predictors of TL. No significant results were found for stress (CTQ ($\beta = .060$ and ELS $\beta = -.009$, $p > .005$) (Supplementary Table 4)).

Association between drinking behavior and TL

Partial correlations between drinking behavior (heavy drinking days, drinks per weeks, heavy drinking years, LDH, AUDIT, ADS) and TL did not show significant associations, neither in AUD or HC (all $p > 05$), corrected for age, gender, BMI, and years of education. The linear regression model showed a significant effect of drinking history on TL for HC ($\beta = .137$, $p = 0.034$), but not for AUD ($\beta = .008$, $p = 0.922$) (Supplementary Table 5). However, after excluding $n = 195$ individuals to match groups regarding age, gender, BMI, years of education, Fagerstrom score, and African ancestry, the effect of drinking history in HC ($n = 127$) did not remain significant ($\beta = .173$, $p = 0.213$).

Association between lifetime stress, trauma, and TL

Partial correlations between childhood lifetime stress (CTQ total score and ELS questionnaire), negative events in adult lifetime (LEQ), and TL did not show significant associations, neither in AUD or HC, corrected for age, gender, BMI, and years of education. Moreover, a two-way ANOVA revealed that early life stress experience (ELSE) assessed by CTQ total score did not significantly influence TL in HC (F (1, 316) = ·35, $p = 0.5$), and there were no differences ($p > 0.5$) in TL between HC without ELSE ($n = 222$) versus HC + ELSE ($n = 10$) (Fig. 2). Similarly, there was no difference in TL between AUD without ELSE ($n = 68$) versus AUD + ELSE ($n = 24$) (Fig. 2).

Table 3 Summary of regression analyses for telomere length (TL) and covariates predicting TL in both the AUD and control group combined ($n = 709$). Significance levels in italics: $^{***}p < .001$

	Telomere length		
Variable	*B*	*SE B*	*β*
AUD	− .167	.052	*− .424****
Age	− .004	.001	*− .277****
AUD vs age	.003	.001	*.322***
Sex	.015	.014	.038
BMI	.001	.001	.029
Education years	.000	.002	− .005
Fagerstrom score	.002	.004	.020
African ancestry	− .011	.020	− .024
R^2	.081		
F	*7.514****		

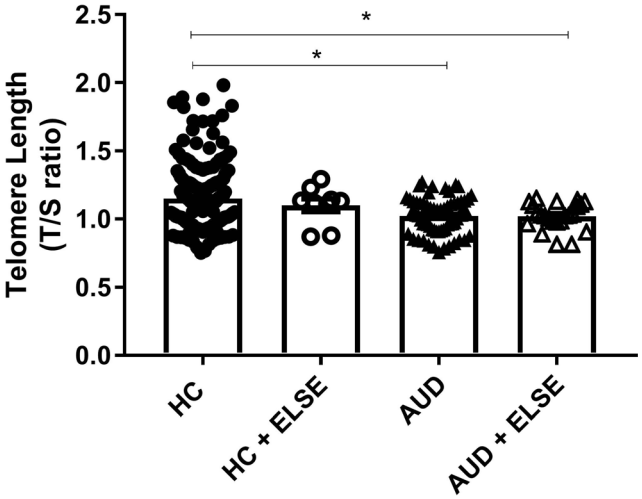

Fig. 2 Comparison of telomere length among HC and AUD participants that experienced early life stress (ELSE) or not. Mean and SE values: HC (1.115 ± .001, $n = 222$), HC + ELSE (1.133 ± .004, $n = 10$), AUD (1.031 ± .01, $n = 66$), AUD + ELSE (1.026 ± .02, $n = 23$)

Association between behavioral measures and TL

In AUD participants only, TL was associated with two first-order factors of the BIS scales cognitive instability ($r = 0.210$, $p < 0.001$) and motor impulsiveness ($r = 0.135$, $p < 0.05$) (Supplementary Fig. 1A and 1B); corrected for age, gender, BMI, and years of education. It also correlated with negative urgency ($r = 0.147$, $p < 0.001$), a subscale of UPPS that measures dimensions of impulse behavior (Supplementary Fig. 1C). There were no significant correlations between TL and other behavioral measures.

Discussion

In the present study, we found shorter TL in AUD participants compared to HC. Furthermore, we found evidence for both age and AUD as independent predictors of TL. However, we found no evidence of an association between chronicity of alcohol use and alcohol doses ingested on TL, since neither drinking behavior nor ADS scores were associated with TL in either group. Moreover, different from previous studies on childhood lifetime stress (Hanssen et al. 2017; Coimbra et al. 2017; Li et al. 2017), we did not find evidence for an effect of early life stress on TL neither for AUD nor HC. The reason(s) for this discrepancy is unclear but might reflect the confounds on the assessment and characterization of stress exposures in an individual's life. The frequency of stress events and their impact on an individual are influenced by age and when they occur in a person's life, by gender, ethnicity, education and socioeconomic status (Farah 2017). Thus, the differential contribution and representation in the participants investigated could influence the ability to detect an association between stress and TL. In AUD we also found a positive correlation between TL and dimensions of impulsive behaviors: cognitive instability, motor impulsiveness, and negative urgency.

Previous studies have reported negative effects of substance use on TL, including alcohol, heroin and cocaine (Cheng et al. 2013; Yang et al. 2013; Levandowski et al. 2016). In this context, our findings are consistent with studies that reported shorter telomeres in alcohol abusers relative to healthy controls (Aida et al. 2011; Pavanello et al. 2011; Strandberg et al. 2012; Yamaki et al. 2019). However, despite a wealth of evidence on environmental factors influencing cellular aging, the exact mechanism by which drugs of abuse affect TL is not clear. For alcohol, the strongest hypothesis is that it is driven by oxidative stress (Wu and Cederbaum 2003). The high guanine content of telomeres makes them more susceptible to reactive oxygen species (ROS), which induce mutations, as well as DNA single-strand breaks (Barzilai and Yamamoto 2004; Reichert and Stier 2017). In this way, the incomplete replication problem plus the accumulation of DNA damage may lead to a loss of DNA sequence and accelerate telomere shortening (Barzilai and Yamamoto 2004). Additionally, alcohol consumption can increase the generation of ROS and interfere with DNA's defense mechanism against ROS (Wu and Cederbaum 2003). Recently, Harpaz et al. (2018) reported a reduction in TL shortening in human cells after inhibition of acetaldehyde metabolism, claiming that acetaldehyde, the primary metabolite of alcohol after its oxidation by alcohol dehydrogenase, rather than alcohol itself, is responsible for TL shortening (Harpaz et al. 2018). In the present study, we were unable to investigate mechanisms that could underlie telomere shortening in AUD participants, including alcohol-induced oxidative stress, telomerase availability (Cheng et al. 2013; Reichert

and Stier 2017), and/or neuroinflammatory processes (Olivieri et al. 2015; Kim et al. 2018).

Despite our finding of AUD as an independent predictor of TL, we found no statistical evidence for the effects of alcohol drinking behavior on TL. A recent meta-analysis on alcohol consumption and TL confirmed that four case-control studies reported significant TL associations with alcohol consumption, whereas only 4 out of 20 cross-sectional studies reported correlations between TL and alcohol consumption (Li et al. 2018). The case-control study of Pavanello et al. (2011) found that alcohol abusers drinking > 4 alcoholic drinks per day had shorter TL than those drinking 4 drinks per day, and Strandberg et al. (2012) found that alcohol consumption of > 1 drink/day was associated with shorter TL, which we were not able to replicate in our sample. In contrast, other studies found associations between alcohol consumption and TL, but did not assess alcohol consumption in detail (e.g., only classified ever drinkers versus never drinkers) (Liu et al. 2009, 2011; Aida et al. 2011). Moreover, while cocaine abuse (DSM-IV) was associated with TL in Levandowski et al. (2016) study, there were no associations between TL and age of onset of drug abuse in the cocaine abusers. Overall, comparisons between these studies on drug consumption and TL remain challenging, because studies use a wide variety of drinking cut-offs and categorizations, for example, non-drinkers, social-drinkers, and ever-drinkers (Li et al. 2018), and alcohol consumption in humans remains difficult to measure since it is highly dependent on self-reports. To the best of our knowledge, our NIAAA database is the largest clinically diagnosed AUD sample to evaluate an association with TL reported thus far, and participants underwent a detailed phenotypic characterization of alcohol drinking behavior, including lifetime quantity and frequency (LDH), recent drinking quantity and frequency (TLFB), problematic drinking (AUDIT), and severity of alcohol dependence (ADS). Therefore, the lack of an association between alcohol drinking behavior and TL may suggest that is not the amount of alcohol drinking per se that drives cellular aging and telomere shortening. Specifically, we hypothesize that the failure to see an association could reflect the large variability in the rate of alcohol metabolism and its bioavailability between subjects. Multiple factors influence the rate of ethanol metabolism and its bioavailability including BMI, body composition (fat relative to muscle content), genetic variations of alcohol metabolizing enzymes, gender, age and ethnicity. Indeed, Pavanello et al. (2011), showed that carriers of the common ADH1B*1/*1 (rs1229984) genotype were more likely to be alcohol abusers, while exhibiting shorter TL. Moreover, Shin and Baik (2016), reported a shorter leukocyte TL only among carriers of the mutant alleles (rs2074356 CT and TT) of *ALDH2*. However, further studies are necessary to investigate how genetics interact with alcohol consumption along with age, gender, and ethnicity on TL shortening.

In our study we also explored other factors that have been associated with TL. It is known that alcohol abuse is associated with childhood trauma and lifetime stress experience that also can affect TL (Kang et al. 2017). In our sample, AUD participants had higher scores for childhood stressful events and reported higher negative events in adult lifetime than HC. However, neither childhood nor adult stressful event exposures were associated with TL within the AUD group nor the HC group. This lack of an association is consistent with findings from Küffer et al. (2016) and Glass et al. (2010), who also failed to replicate telomere shortening findings in posttraumatic stress disorder and in childhood maltreatment, respectively. Explanations include possible resilience, a lack of persistence of the effects of child trauma into late life, as well as differences in the questionnaires to assess trauma and in the methodology to assess TL (Glass et al. 2010; Küffer et al. 2016). However, the negative results could also reflect lack of power since our HC sample having exposure to stress events was very small; only 10 participants out of 222, that completed the questionnaire, reported early life stress experiences (score for emotional, sexual, and physical abuse and neglect in CTQ questionnaire, did not reach the cut-off established in Bernstein et al. (1994)). For our AUD sample, the effects of childhood lifetime stress experience may have not been strong enough in relation to the effects of AUD to telomere shortening. Regarding the HC sample, additionally, we need to consider other confounding genetics (Savage and Bertuch 2010; Wei et al. 2015) and environmental (Latifovic et al. 2016) factors as well as BMI (Gielen et al. 2018), which have been associated with TL in previous research. In our sample, AUD participant had higher BMI than HC, and although we covaried for BMI, we cannot rule out a possible interaction between higher BMI and alcohol exposures with TL. However, this is unlikely since BMI was not an independent predictor of TL in our regression analysis in HC or AUD participants. Similarly, while exposure to tobacco has been associated with TL (Marcon et al. 2017) in our sample, Fagerstrom score was associated with AUD, but with TL only at a trend level ($p < 0.1$). Because we do not have data for past exposure to tobacco, we cannot rule out the possibility that the combination of heavy drinking and past smoke exposure might have contributed to shorter telomeres in AUD.

We also explored the association between cognition and TL. Studies have tried to address the role of TL in cognitive impairment; however, despite TL and cognitive ability being correlated in age-adjusted models, this correlation did not survive when other covariates were adjusted, such as socioeconomic status and education (Hägg et al. 2017; Zhan et al. 2018). Similarly, in our study, IQ was strongly correlated with education and age and when correcting for these variables no significant association between IQ and TL was observed. An association between impulsivity and TL was also reported by Kang et al. (2017), who reported that higher delay

discounting, an impulsivity trait characterized by impatience to delays and risk-based decision-making, was associated with shorter leukocyte TL in patients with alcohol dependence and with high levels of childhood maltreatment. Another study performed by Yim et al. (2016) also showed an association between shorter TL and steeper delay discount in a sample of 1158 Han Chinese undergraduates. In our study, despite the use of different measures for impulsive behavior, we also observed an association between TL and motor impulsiveness, urgency, and cognitive instability, which are also core endophenotypes of impulsiveness.

A limitation in our study is that the cross-sectional design allowed us to evaluate the TL in only one point of time; thus, the results can only be interpreted as correlational and further work is required to establish causality between alcohol abuse and shorter telomeres and to assess whether TL could be used as a biomarker for drinking.

In summary, the present results show that both AUD and age independently predict shorter TL, but the interaction of age and AUD is also associated with TL. Their independence and interaction modulating TL in AUD are consistent with alcohol acceleration of cellular aging.

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Authors' contribution All authors discussed the results and contributed to the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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