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The association of telomere length with substance use disorders: a systematic review and meta-analysis of observational studies

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ABSTRACT

Background and Aims Several recent studies have investigated the relationship between telomere length and substance use disorders with inconsistent results. We aimed to assess this association and to identify moderators of the relationship. **Methods** Systematic review and meta-analysis. Selection criteria were observational studies reporting telomere length in people with a substance use disorder compared with a control group. Studies focused solely on nicotine addiction, employing other study designs, and non-human studies were excluded. Study selection and data extraction were independently conducted by two researchers following a standardized protocol and included studies until December 2019. Standardized mean differences were used as the effect size index [d; 95% confidence interval (CI)] and random-effects models were used for the meta-analysis. Cochran's Q-statistic, I^2 index, visual inspection of the forest plot and a 95% prediction interval were applied to verify study heterogeneity. Subgroup analyses and meta-regressions were conducted to explore heterogeneity. Small study effects were examined using the 'funnel plot', the Egger test, Duval & Tweedie's trim-and-fill method and the precision-effect test–precision-effect estimate with standard error (PET-PEESE) method. The risk of bias and the quality of evidence were assessed. **Results** Ten studies (12 analysis units with 2671 cases and 4532 controls) met the selection criteria. An overall effect size of moderate magnitude was found ($d_+ = -0.63$; 95% CI = −1.00 and −0.26; $P = 0.0008$). A potential small study effect was detected, as well as large heterogeneity between studies (Q-statistic $P < 0.001$, $I^2 = 97.3\%$). Selection of controls, reporting laboratory quality control procedures and total sample size significantly affected the effect size. The quality of the evidence was very low, based on risk of bias analysis and the grading of recommendations assessment, development and evaluation (GRADE) system. **Conclusions** People with substance use disorders appear to have shorter telomere length than controls; however, this finding should be interpreted with caution due to the poor quality of the evidence.

Keywords Alcohol, cellular ageing, meta-analysis, substance use disorders, systematic review, telomere length.

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INTRODUCTION

Telomeres are repetitive non-coding DNA protein structures consisting of nucleotide sequences of tandem TTAGGG repeats at the end of chromosomes in association with a protein complex. These structures are essential for maintaining genome stability [1] and for ensuring the regulation of gene expression [2]. Telomere length (TL) varies throughout the life-span and is considered to be a marker of cellular ageing [3–5]. Telomere attrition has been associated with increased all-cause mortality risk [6], and in particular with increased morbidity of various age-related diseases [7–12]. Results of recent meta-analyses suggest that TL might be associated with a variety of mental disorders [13–19]. However, a non-systematic review has highlighted inconsistencies of the published results regarding the association between substance use disorders (SUDs) and telomere length [20].

SUDs constitute one of the major public health issues around the world [21,22], and are major contributors to burden of disease [23] with greater risk of disability [24] and mortality [25]. Early detection of addiction is considered crucial for preventing premature morbidity and mortality [26]. Comorbidity is highly prevalent between SUDs

and both psychiatric disorders [27] and medical conditions [28]. To the best of our knowledge, no systematic review or meta-analysis examining the association of telomere length with SUD related to any substance other than tobacco [29] was ever conducted.

The aims of the present study were (i) to determine whether people with SUDs have shorter telomere lengths compared to healthy controls, (ii) to explore potential differential effects with regard to diverse substances and (iii) to identify potential moderators of the telomere length effect. The research questions were: (i) do people with SUDs have shorter telomere lengths compared with healthy controls; (ii) are there differences in the association of TL with SUD as a function of the type of substance that is misused; and (iii) if heterogeneity is confirmed, what are the factors implicated?

METHODS

Protocol and registration

The protocol of this investigation was registered with the International Prospective Register of Systematic Reviews (PROSPERO 2019 CRD42019119785, https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=119785) and published previously [30]. We wrote this report using the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA guidelines) [31] and the proposal for reporting Meta-analysis of Observational Studies in Epidemiology (MOOSE) [32].

Study eligibility criteria

Inclusion criteria were as follows—(a) populations: adults with SUDs, except if the disorder is exclusively based on nicotine addiction, and healthy controls; and (b) exposure: SUD covered alcohol, illicit drugs including cocaine, opiates or other substances (e.g. marijuana and amphetamine, among others). Case status had to be defined as having any SUD identified through a clinical interview or using established standard diagnostic instruments including, but not limited to, the Structured Clinical Interview for DSM-IV (SCID), Computerized National Institute of Mental Health Diagnostic Interview Schedule (CDISIV), the Composite International Diagnostic Interview (CIDI) or any other diagnostic instrument based on Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria or (c) control group: adults with no SUD diagnosis (e.g. from the general population, the community, unexposed outpatient or hospital-based controls); (d) outcomes: telomere length measurements with a detailed description of both the methods of measurement and the isolated tissue that was used; and (e) study designs: observational studies (case–control, cohort, cross-sectional, longitudinal designs). Exclusion criteria were: systematic or narrative reviews, meta-analyses, studies with non-human samples or other designs including reviews, case-only studies, family-based designs and population-based studies with healthy subjects only, as well as studies focused on tobacco smoking.

Information sources and search strategy

Comprehensive electronic searches were conducted to identify studies indexed in PubMed/MEDLINE, EMBASE, Psychlit/PsychINFO and Web of Science databases (Web of Knowledge) from inception until December 2019. The search was performed by a librarian with expertise in systematic reviews. The following search terms were used for SUDs: 'drug, substance, addiction, alcohol*, heroin, cocaine, opium, opioid, methamphetamine, morphine' and for telomeres: *Telomeres, telomerase, and telo**' (see Supporting information, Table S1). The references cited in each study included in this initial selection and in review articles were then manually searched to identify other potentially eligible studies. To minimize potential publication bias, both published and unpublished papers were eligible for inclusion. In order to identify unpublished studies, e-mails were sent to the corresponding authors of the selected studies to enquire about any potential study that met eligibility criteria. In the search strategy, no restrictions were placed on time-period, sample size, ethnicity or language of publication.

Data extraction

The following data were extracted from each study following the previously defined protocol: (i) identification data of the study (author(s), journal, language and year of publication); (ii) methods (study design, sample sizes for both cases and controls, diagnostic tools for the determination of case status, definition of case status, variables adjusted for in the analyses, attrition for cases and controls and differential attrition); (iii) risk of bias assessment (described in greater detail below); (iv) sample characteristics for both cases and controls separately [gender ratio, mean age and standard deviation (SD), ethnic background, education level, type of substance used in SUD cases, duration of SUD in cases, presence of comorbid mental disorders or medical conditions in cases, smoking status, exposure to childhood adversities and other stressful life events]; (v) telomere-related information (telomere length, tissue source and telomere measurement method) and (vi) extrinsic characteristics (relevant ethical approval, conflict of interest disclosure and funding source).

If an article reported two or more studies with independent samples, then each independent study was included as an analysis unit in the meta-analysis. When essential data were unavailable in the original studies, authors of the respective papers were contacted and asked to provide additional data. Two reviewers independently determined eligibility and extracted data from included studies. Disagreements were resolved by consensus or reached with the involvement of a third reviewer. To assess the reliability of the data extraction process in terms of inter-rater agreement, kappa coefficients were calculated between the two reviewers.

Risk of bias assessment

The risk of bias of each included study was assessed using the Newcastle–Ottawa Scale (NOS [33]). Discrepancies in the quality assessment of each study were resolved by consensus. A total quality score (TQS) of each individual study was calculated by adding all the stars (range = 0–9, with a higher score indicating higher overall quality). Studies were not weighted by the TQS and the influence on the effect size of each item was individually assessed [34]. In addition to the NOS, several study characteristics (e.g. if a blind assay assessment and genetic quality procedures were reported, as well as the evaluation of psychiatric or physical comorbidities or the exposure to childhood adversities or other stressful events) were extracted to analyse their potential risk of bias on the effect sizes.

Effect size index

For each study, means and SDs on TL measured in the T/S ratio scale were extracted. These data were converted into Hedges' standardized mean difference (*d*) as effect size index. The *d* index was calculated as the mean difference in telomere length between the SUD and control groups, divided by the pooled standard deviation of the two groups [35]. Negative *d*s represented a shorter telomere length for the SUD group compared to the control group. By convention, *d* indices of 0.20, 0.50 and 0.80 (in absolute value) were considered to be of small, moderate and large magnitude, respectively [36]. For each *d* index, a 95% confidence interval (95% CI) was calculated. In this meta-analysis unadjusted effect sizes (*d*s) were used. As described in the Results section, the reason for not analysing adjusted effect sizes was that the majority of the studies did not report the statistical information needed to calculate an adjusted effect size using the same metric used for the unadjusted standardized mean difference (i.e. adjusted means and SDs to calculate adjusted standardized mean differences). The potential influence of confounding factors was assessed as described below. Supporting information, Table S2 describes how the data were extracted from the studies and *d* indices were calculated.

Statistical analyses

andom-effects models were used to analyse the TL–SUD association due to an expectation of a high level of heterogeneity among the studies. An average effect size and a 95% CI was calculated with the improved method proposed by Hartung & Knapp [37–39]. In addition, a 95% prediction interval around the average effect size was calculated in order to provide a prediction of the expected true effects if a new study is conducted [40].

To estimate heterogeneity between studies, the Cochran's *Q*-statistic, the I^2 index and visual inspection of the forest plots were used. In addition, heterogeneity was assessed with the between-studies variance and corresponding 95% confidence interval [41]. Finally, the estimated proportion (and 95% CI) of true effect sizes exceeding a meaningful threshold was calculated, considering −0.20 as the threshold effect size for these calculations in terms of standardized mean difference [42].

In cases of moderate-to-large heterogeneity ($I^2 > 25\%$), we attempted to identify possible explanations using subgroup analyses and meta-regressions based on the most important characteristics of the studies, including items used to evaluate the risk of bias. The analyses of moderating variables were individually assessed [32] and were accomplished by assuming a mixed-effects model [43]. The improved *F*-statistic was applied for testing the statistical significance of each moderator [44]. To estimate the proportion of variance accounted for by the moderator, an R^2 index was calculated [45]. Simple and multiple mixed-effects meta-regression was applied to analyse the influence of the following moderators on the effect sizes: publication year, mean and SD of the age (total, case and control samples), mean age difference, SD of age difference, percentage male (total, case and control sample), percentage male difference, sample size and NOS total quality score.

The presence of small study effects was examined using the 'funnel plot' method in combination with Duval & Tweedie's trim-and-fill method [46], the Egger test [47] and the precision-effect test–precision-effect estimate with standard error (PET-PEESE) method [48]. An additional sensitivity analysis was performed with the 'leave-one-out' method, by systematically removing each study and re-calculating the overall results. All statistical analyses were conducted using the metafor program in R [49], except for the PET-PEESE method that was conducted with SPSS macros [48]. The grading of recommendations assessment, development and evaluation (GRADE) approach was used to evaluate the quality of evidence [50].

RESULTS

Study eligibility and data collection

We first identified a total of 1173 studies. After duplicates were removed, titles and abstracts of 701 studies were screened for eligibility and 558 were excluded. A total of 143 full-text studies were assessed for eligibility and 133 were excluded (see flow-chart in Fig. 1 and individual reasons for exclusion in Supporting information, Table S3). Inter-rater agreement in the selection process was reached in 96% of the studies. Finally, 10 studies (12 analysis units) were selected for the meta-analysis. Although efforts to identify unpublished studies were made, all the studies included in this meta-analysis were published articles. The main characteristics of these studies are summarized in Tables 1 and 2. The median (SD) of the Cohen's kappa inter-rater agreement coefficient was 0.70 (0.24) and ranged from 0.16 to 1.00.

The 10 eligible studies included 7203 participants (2671 cases and 4532 controls). As shown in Table 1, all studies applied a case–control design. The most represented countries were the United States with three studies [51–53] and Japan with two studies [54,55]. Case samples presented mean ages ranging between 26.2 and 74.5 years (mean = 47.4), whereas control samples ranged from 33.3 to 75.1 (mean = 55.1). Three studies included men only [55–57] and one study with two analysis units included women only [58]. Related to the type of substance used, five studies investigated alcohol [52–55,57], one alcohol and cocaine [51], one cocaine [58], one tobacco and marijuana [56], one a mixture of cocaine, heroin, methamphetamine and morphine [59] and one study did not describe the substances consumed by those diagnosed with SUD [60].

Adjusted effect sizes were not calculated, as the majority of the studies did not report the statistical data needed to obtain adjusted standardized mean differences [51–53,58–60]. In addition, in one study this information was reported [57], but in terms of geometric means and not as arithmetic means, and two studies did not apply adjusted analyses [54,56]. Only one study [55] reported statistical data needed to calculate an adjusted standardized mean difference, with a value of $d_{adj} = -1.81$ (95% CI = −2.10 and −1.52), which was very similar to the

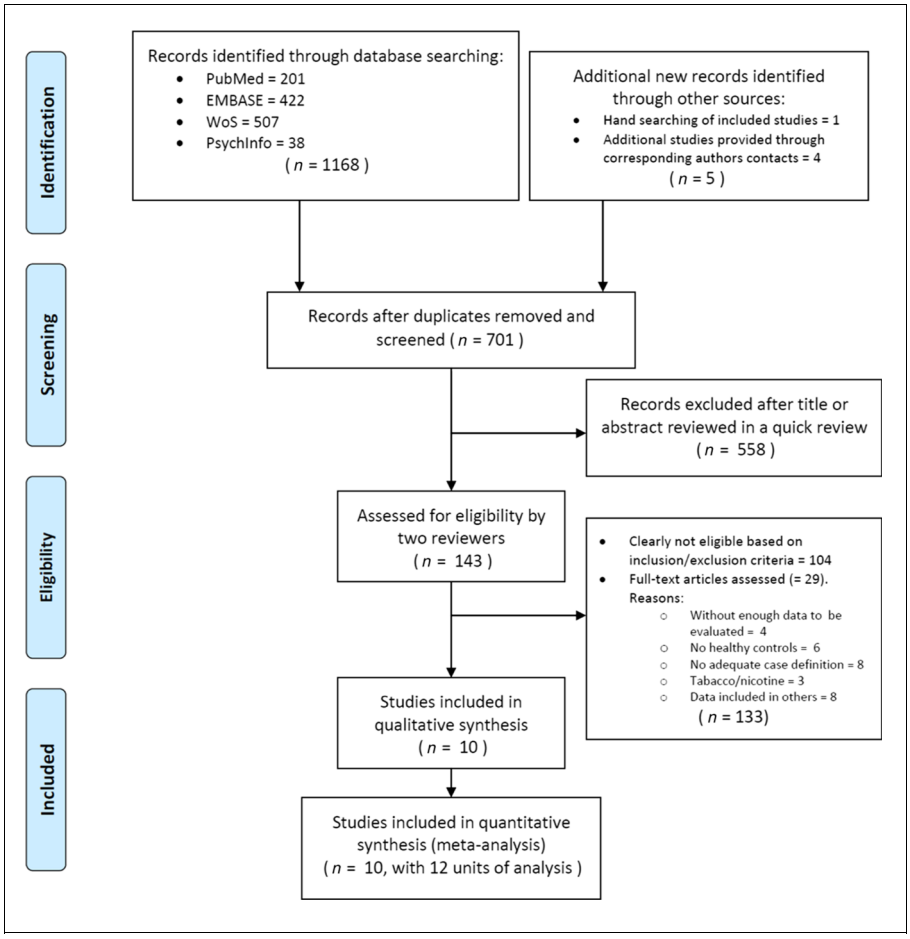

Figure 1 Flow-chart of the meta-analysis of telomere length and substance use disorders. Adapted from Moher *et al.* [31].

Table 1 Main characteristics of included studies

			Cases		*Controls*		*Age*				
First author, year	*Country*	*Study design*	*N*	*Males n (%)*	*N*	*Males n (%)*	*Cases, mean (SD/range)*	*Control mean (SD/range)*	*Substance*	*SUD[a] instrument*	*Overall association*
Aida, 2011	Japan	Case–control	26	26	24	12	61.2 (44–82)	73.3 (41–95)	Alcohol	DSM-IV	Shorter TL in the oesophageal epithelium of cases
Pavanello, 2011	Italy	Case–control	200	200	257	257	38 (35–75)	44 (25–62)	Alcohol and smoking	DSM-IV-TR	Shorter TL in alcohol abusers compared to controls
Savolainen, 2012	Finland	Nested case–control[b]	40	0	1840	842	–	61.5 (2.9)	Not described	Finnish Hospital Discharge Register (ICD-9 and 10 and DSM-III-R classification systems)	Participants hospitalized for any mental or substance use disorders had longer TL than non-hospitalized controls
Yang, 2013	China	Case–control	415	199	508	210	33.79 (7.6))	34.46 (8.16)	Heroin, morphine, methadone, tramadol, marijuana, MDMA, methamphetamine, ketamine, triazolam	Standardized questionnaires and protocols not described	Drug abusers exhibited significantly shorter TLs than controls
Mohamed, 2016	Egypt	Case–control	30	30	NS[c] = 30 S[c] = 30	NS[c] = 30 S[c] = 30	41.83 (6.94)	NS[c] = 40.53 (7.52) S[c] = 41.07 (6.98)	Tobacco and marihuana	Not described[d]	TL was shorter in marijuana smokers' group than in smokers or non-smokers' groups
Levandowski, 2016	Brazil	Case–control	34	0	49	0	26.2 (6.3)	68.3 (7.4)	Cocaine	Clinical interview and semi-structured clinical interview following DSM-IV criteria, CSSA, ASI-6	TL is shorter in crack with/without early life stress than in controls
Tannous, 2019	USA	Case–control	24	18	25	17	46.96 (7.66)	43.76 (6.62)	Alcohol and cocaine	SCID-IV, ASI, KMSK	No significant TL differences in comorbid

(***Continues***)

Table 1. (Continued)

First author, year	Country	Study design	Cases N	Cases Males n (%)	Controls N	Controls Males n (%)	Age Cases, mean (SD/range)	Age Control mean (SD/range)	Substance	SUD[a] instrument	Overall association
											cocaine and alcohol use disorder
Yamaki, 2019	Japan	Case–control	134	134	121	121	58.7 (9.7)	59 (10.2)	Alcohol	Kurihama Alcoholism Screening Test and DSM-IV criteria	TL was almost 50% shorter in patients with alcohol dependence (AD) compared to controls
Martins, 2019	USA	Case–control	260	187	449	248	44.06 (0.73)	33.32 (0.56)	Alcohol	SCID, AUDIT, ADS	TL is shorter in participants with alcohol use disorders compared to healthy controls
Dixit, 2019[e] (A)	USA	Nested case–control[b]	627	527	321	246	67.1 (10.8)	66.1 (11.2)	Alcohol	AUDIT-C	No association between TL and alcohol consumers
(B)	USA	Nested case–control	790	376	883	314	74.5 (5)	75.1 (5.5)	Alcohol	Self-reported	No association between TL and alcohol consumers

[a]SUD = substance use disorder; AUDIT = Alcohol Use Disorders Identification Test; ICD = International Statistical Classification of Diseases and Related Health Problems; DSM = Diagnostic and Statistical Manual of Mental Disorders; CSSA = Cocaine Selective Severity Assessment; ASI-6 = Addiction Severity Index version 6; SCID = Structured Clinical Interview for DSM-IV; KMSK = Kreek–McHugh–Schluger–Kellogg scale; ADS= Alcohol Dependence Scale; TL = telomere length; SD = standard deviation; MDMA = 3,4-methylenedioxymethamphetamine. [b]Only 5-year follow-up measurement of telomere length in cohorts was included in the analyses. [c]NS = non-smokers; S = smokers. [d]Most participants were recruited from a hospital. It was confirmed by measurement of delta-9-tetrahydrocannabinol (THC) in urine samples. [e]Dixit *et al* (2019) describes results of two other independent studies: (A) The Heart and Soul Study and (B) The Cardiovascular Health Study (B).

Table 2 Telomere measurement description in included studies

First author, year	Cases telomere length (T/S ratio)		Controls Telomere length (T/S ratio)		Original TL measurement	Technique used	Tissue source	Blind assay assessment reported	Genetic quality control	Genetic quality control description
	Mean	SD	Mean	SD						
Aida, 2011	1.22	0.6	1.64	0.6	Normalized telomere-to-centromere ratio (NTCR)	Q-FISH	Esophageal mucosa (basal and parabasal cells)	NR	Yes	Control for variations in sample preparation
Pavanello, 2011	0.45	0.12	1.06	0.82	T/S ratio	Multiplex real-time qPCR	Leucocytes	Yes	Yes	All samples run in triplicate and the average of the three T/S ratio measurements was used. Repetition of the assay for 20 samples in two different ways
Savolainen, 2012	NR	NR	NR	NR	T/S ratio	Real-time qPCR	Leucocytes	NR	Yes	Triplicates with amplification curve standard deviations above 0.5 at the threshold level were omitted
Yang, 2013	0.78	0.18	0.84	0.21	T/S ratio	qPCR	Leucocytes	NR	Yes	All samples were run in duplicate and evaluated correlation. Samples with a CV > 2% were excluded and re-run. To test the reproducibility of the assay, multivariate samples were randomly chosen and run again.
Mohamed, 2016	0.61	0.09	0.77	0.11	T/S ratio	qPCR	Leucocytes	NR	Yes	In duplicate 10-ml reaction within the same plate and calibrator genomic DNA in each run of the samples
Levandowski, 2016 (A)	1.33	0.16	1.5	0.42	T/S ratio	qPCR	Peripheral blood	NR	Yes	Prior to the experiment, primer
(B)	1.19	0.21	1.5	0.42	T/S ratio	qPCR	Peripheral blood	NR	NR	sets were tested thoroughly to determine reaction efficiency,

(*Continues*)

Table 2. (Continued)

First author, year	Cases telomere length (T/S ratio)		Controls Telomere length (T/S ratio)		Original TL measurement	Technique used	Tissue source	Blind assay assessment reported	Genetic quality control	Genetic quality control description
	Mean	SD	Mean	SD						
										specificity, and the absence of primer-dimers
Tannous, 2019	0.93	0.34	1.13	0.70	T/S ratio	PCR	Leucocytes	NR	Yes	DNA samples run in duplicate
Yamaki, 2019	1.49[a]	0.626	3.96[a]	1.778	kbp[a]	Telo TAGGG telomere length assay kit	Leucocytes	NR	NR	–
Martins, 2019	1.06	0.00	1.14	0.01	T/S ratio	Monochrome multiplex qPCR	Whole blood	NR	Yes	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
Dixit, 2019 (A)	0.83[a]	0.149	0.84[a]	0.145	kpb[a]	qPCR	Leucocytes	NR	Yes	T/S ratio measured in duplicate and the averaged was used for each participant
(B)	1.26[a]	0.249	1.27[a]	0.261	kbp[a]	Southern blot analysis of terminal restriction fragment lengths	Leucocytes	Yes	Yes	Telomere measurements were performed in duplicate

kbp = kilobase pairs; qPCR = quantitative polymerase chain reaction; Q-FISH = quantitative fluorescence *in-situ* hybridization; NR = not reported; CV = coefficient of variation; SD = standard deviation. [a]Base pairs (bp) were transformed to T/S ratio using the formula: bp = 3274 + 2413 × (T/S) (Dixit *et al*, 2019).

Table 3 Quality characteristics of included studies

	New Newcastle–Ottawa Scale for case–control studies								
	Selection				*Comparability*	*Exposure*			
First author, year	*(1) Case definition*	*(2) Representativeness of cases*	*(3) Selection of controls*	*(4) Definition of controls*	*(1) Comparability*	*(1) Ascertainment of exposure*	*(2) Same method of ascertainment*	*(3) Non-response rate*	*NOS score*
Aida, 2011	*	–	–	–	–	–	–	–	1/9
Pavanello, 2011	*	–	–	*	**	–	*	–	5/9
Savolainen, 2012	*	*	*	*	**	–	*	–	7/9
Yang, 2013	–	–	–	*	**	–	–	–	3/9
Mohamed, 2016	–	–	–	*	**	*	*	–	5/9
Levandowski, 2016	*	–	–	*	**	–	*	–	5/9
Tannous, 2019	*	–	*	–	**	–	*	–	5/9
Yamaki, 2019	–	–	*	*	**	–	–	–	5/9
Martins, 2019	*	–	*	*	**	–	*	–	6/9
Dixit, 2019 (A)	*	–	*	*	**	–	*	–	6/9
(B)	–	–	*	*	**	–	*	–	5/9

Table 3. (Continued)

	Evaluate…				*Control for (matched or controlled)*[a]			
First author, year	*Psychiatric comorbidity (yes/no)*	*Physical comorbidity (yes/no)*	*Childhood adversities (yes/no)*	*Other stressful event exposure (yes/no)*	*Age*	*Sex*	*Smoking*	*Other covariates controlled*
Pavanello, 2011	No	No	No	Yes	Yes (C)	Yes (M)	Yes (C)	BMI, vegetable intake, and jobs with elevate risk of accident
Savolainen, 2012	Yes	Yes	No	No	Yes (C)	Yes (C)	Yes (C)	Diabetes mellitus, BMI, alcohol consumption and coronary heart disease
Yang, 2013	Yes	Yes	No	Yes	Yes (C)	Yes (C)	Yes (M)	Participant self-assessment indicated that all of them were free of serious illness (infectious disease, cardiovascular diseases, mental disorders and cancer)
Mohamed, 2016	Yes	Yes	No	No	Yes (M)	Yes (M)	Yes (M)	Socio-economic status (M)
Levandowski, 2016	Yes	Yes	Yes	No	Yes (C)	Yes (M)	No	BMI and education level
Tannous, 2019	No	No	No	No	Yes (C)	No	No	Education
Yamaki, 2019	No	Yes	No	No	Yes (C)	No	Yes (C)	Alcohol consumption, and cancer
Martins, 2019	Yes	No	Yes	Yes	Yes (C)	Yes (C)	Yes (C)	BMI, years of education, and African ancestry
Dixit, 2019 (A)	No	Yes	No	No	Yes (C)	Yes (C)	Yes (C)	Race, BMI, waist–hip ratio, number of pack years and a group of medical conditions[b] and inflammatory markers and omega-3 fatty acid levels
(B)	No	Yes	No	No	Yes (C)	Yes (C)	Yes (C)	

BDI = Beck Depression Inventory; MHI = Mental Health Index; VS = Vitality Scale; BMI = body mass index; CTQ = Childhood Trauma Questionnaire; ELS = Early Life Stress Questionnaire; NR = not reported. [a]The statistical analyses applied in each study are described in Supporting information, Table S2. (M) = control by matching; (C) = statistical control. [b]Medical conditions: diabetes, hypertension, coronary artery disease, prior myocardial infarction, heart failure, prior stroke and liver disease.

unadjusted d index: $d = -1.89$ (95% CI = −2.18 and −1.59). As shown in Table 3, the variables most frequently used to adjust the SUD-TL association were the age, sex and smoking status. The results of the adjusted statistical analyses reported in the studies (multiple linear regression models in most cases) are described in Supporting information, Table S3. Therefore, meta-analytical calculations were based on unadjusted effect sizes.

Average effect size and heterogeneity

A forest plot of the d indices comparing average telomere length of SUD and control samples is presented in Fig. 2. With one exception [60], every study exhibited shortened telomere length in SUD samples in comparison with controls, with eight studies reaching statistical significance [52,54–60]. An overall effect size of moderate magnitude was found ($d_+ = -0.63$; 95% CI = −1.00 and −0.26; $P = 0.0008$). The 95% prediction interval (−2.06 to 0.80) was wide, indicating that the expected effect size in a new study could exhibit a wide range of true effect sizes, both of negative or positive sign. It was estimated, taking into account the overall effect size and the between-studies variance, that approximately 75.5% (95% CI = 54.6%, 96.4%) of true effect sizes exceeded the threshold for a scientifically meaningful size of $d = -0.20$. In addition, taking $d = 0.20$ as a threshold in the inverse direction, this method estimated that only 9.2% of true effect sizes exceeded that threshold (95% CI = 0%, 23%). As a sensitivity analysis, the 'leave-one-out' method was applied, finding three studies whose exclusion led to a change larger than 10% in the overall effect size (d_{-1} values = −0.56 [56]; −0.50 [55]) and −0.72 [60]), but in all cases the adjusted overall effect size was statistically significant and of moderate magnitude ($d > |0.50|$).

The Q-statistic to assess heterogeneity among the effect sizes was statistically significant (Q [11] = 256.56, $P < 0.001$) and the I^2 index was of large magnitude ($I^2 = 97.3\%$), as well as the between-studies variance ($\tau^2 = 0.39$; 95% CI = 0.04, 0.74). Taken together, these findings revealed the existence of large heterogeneity between studies.

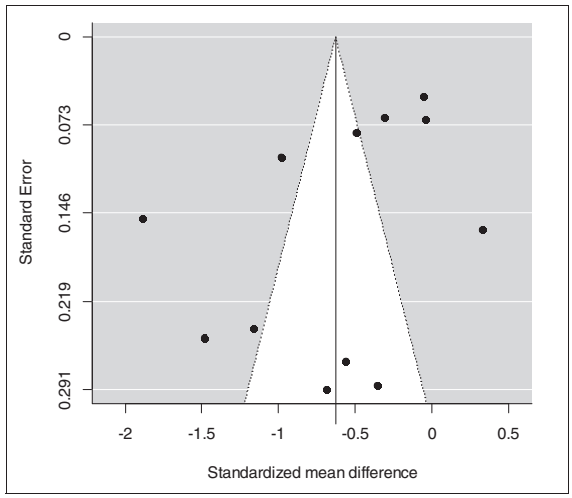

Figure 3 Funnel plot of the 12 standardized mean differences comparing average telomere length of substance use disorder (SUD) and control samples

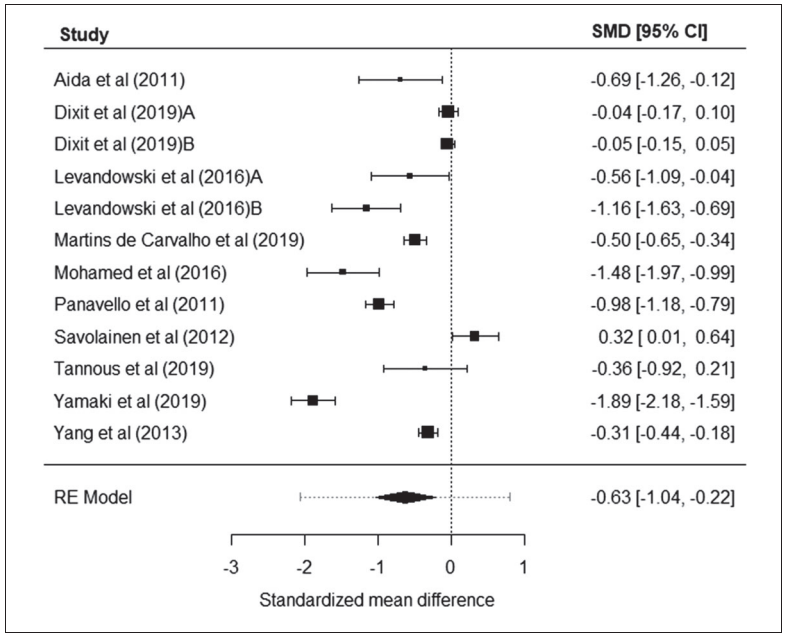

Figure 2 Forest plot of the standardized mean differences comparing average telomere length of substance use disorder (SUD) and control samples. SMD = standardized mean difference

Small study effect analyses

To assess whether small study effects were affecting to the meta-analytical results, a funnel plot was constructed as reported in Fig. 3. The existence of asymmetry in the funnel plot was corroborated with the Egger test, that reached statistical significance (t [10] = − 1.93, P = 0.082). The trim-and-fill method to symmetrize the funnel plot did not add to the effect size. However, when the PET-PEESE method was applied, an estimate of the overall effect size adjusted by small study effects was of practically null magnitude (d_{PET} = 0.05; 95% CI = −0.46, 0.56).

Risk of bias analyses

The methodological quality of the studies was assessed with the NOS, together with several additional items not included in NOS (see Table 3 and Fig. 4). According to the GRADE system [50], there is very low-quality evidence that people with SUDs have shorter TL (see Supporting information, Table S4, based on Cochrane's template for assessing the GRADE criteria [61]).

The potential relationship between each item of NOS and the effect sizes was assessed by means of subgroup analyses (see Table 4). There was some evidence for the effect size varying by the selection of controls (P = 0.016; R^2 = 0.43). Studies that selected controls from a hospitalized population or with no description of the selection process exhibited a slightly higher but non-statistically significant average effect size compared to those with community controls (d_+ = −0.94 versus −0.07). Table 4 presents the results of subgroup analyses for three additional methodological characteristics. Of these analyses, the only one that exhibited a relevant association with the effect sizes was whether the study reported quality control procedures in genotyping methods (P = 0.028; R^2 = 0.37), such that a lower average effect size was found when quality control methods were applied than when they were not reported (d_+ = −0.50 versus −1.88). However, this result must be interpreted cautiously, because only one study did not report quality control methods.

Types of substances related to SUD and telomere length

Studies were classified in three categories as a function of the type of substance misuse: alcohol, other substances (mainly cocaine) and alcohol plus cocaine. Table 5 presents the results of comparing the average effect sizes for these three categories. No relevant differences were found between the three types of substance (P = 0.788; R^2 = 0). An additional analysis consisted of defining three dichotomous variables to categorize studies included consumers of alcohol, cocaine and other substances, with codes 0 (no consumers of that substance) and 1 (consumers). Then, a multiple meta-regression analysis was applied with these three moderators and the effect

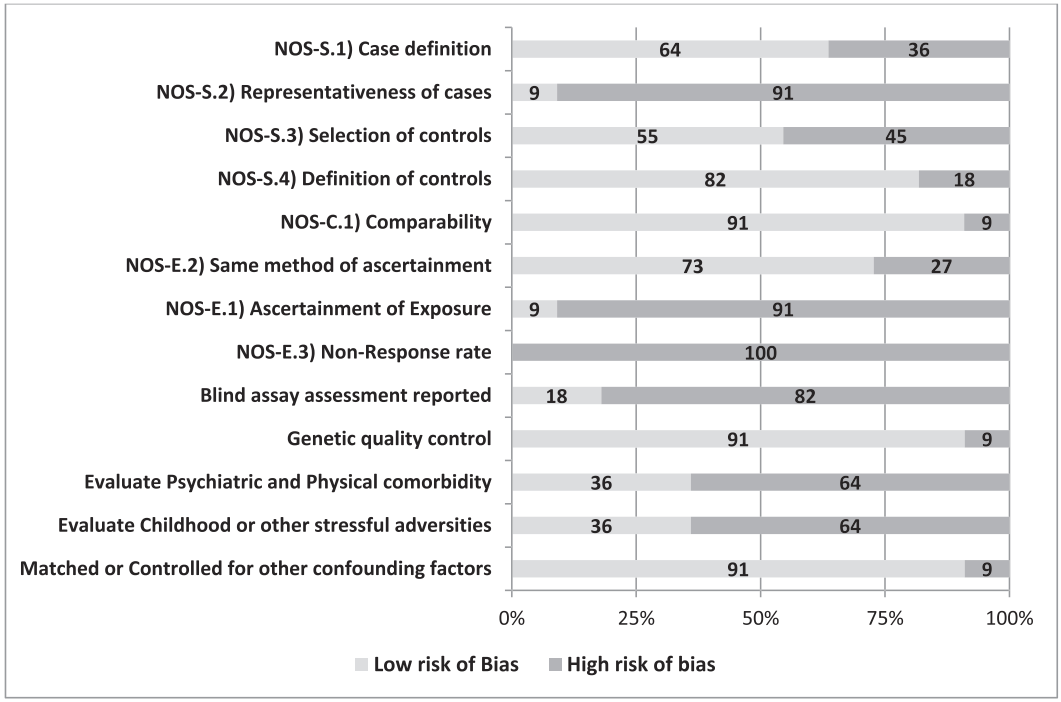

Figure 4 Risk of bias assessment of included studies. NOS: Newcastle–Ottawa Scale for case–control studies. NOS-S: selection; NOS-C: comparability; NOS-E: exposure

Table 4 Results of the subgroup analyses for the Newcastle–Ottawa Scale (NOS)[a] and methodological characteristics on the effect sizes

				95% CI		
Moderator variable	*N*	*k*	d_+	d_L	d_U	*ANOVA results*
(NOS-S1) Case definition						
No	4814	5	−0.66	−1.32	0.004	$F_{(1,10)} = 0.02$, $P = 0.896$
Yes	2389	7	−0.61	−1.18	−0.03	$R^2 = 0$ Q_W (10) = 246.71, $P < 0.001$
(NOS-S2) Representativeness of cases						
No	5323	11	−0.72	−1.12	−0.31	$F_{(1,10)} = 2.84$, $P = 0.123$
Yes	1880	1	0.32	−0.99	1.64	$R^2 = 0.14$ Q_W (10) = 239.53, $P < 0.001$
(NOS-S3) Selection of controls						
No	1993	8	−0.94	−1.34	−0.53	$F_{(1,10)} = 8.31$, $P = 0.016$
Yes	5210	4	−0.07	−0.60	0.46	$R^2 = 0.43$ Q_W (10) = 152.54, $P < 0.001$
(NOS-S4) Definition of controls						
No	50	1	−0.69	−2.27	0.90	$F_{(1,10)} = 0.01$, $P = 0.933$
Yes	7153	11	−0.62	−1.08	−0.17	$R^2 = 0$ Q_W (10) = 254.98, $P < 0.001$
(NOS-C1) Comparability						
No	50	1	−0.69	−2.27	0.90	$F_{(1,10)} = 0.01$ $P = 0.933$
Yes	7153	11	−0.62	−1.08	−0.17	$R^2 = 0$ Q_W (10) = 254.98, $P < 0.001$
(NOS-E1) Ascertainment of exposure						
No	7113	11	−0.56	−0.97	−0.14	$F_{(1,10)} = 1.91$, $P = 0.196$
Yes	90	1	−1.48	−2.91	−0.05	$R^2 = 0.08$ Q_W (10) = 234.82, $P < 0.001$
(NOS-E2) Same method of ascertainment						
No	1221	3	−0.96	−1.79	−0.14	$F_{(1,10)} = 1.09$, $P = 0.321$
Yes	5982	9	−0.52	−0.99	−0.04	$R^2 = 0.01$ Q_W (10) = 233.99, $P < 0.001$
Controls without SUD?[b]						
No	2729	4	−0.60	−1.36	0.15	$F_{(1,10)} = 0.01$, $P = 0.925$
Yes	4474	8	−0.64	−1.17	−0.11	$R^2 = 0$ Q_W (10) = 228.51, $P < 0.001$
Blinded assessors?						
Not reported	5073	10	−0.65	−1.13	−0.18	$F_{(1,10)} = 0.08$, $P = 0.785$
Yes	2130	2	−0.51	−1.54	0.51	$R^2 = 0$ Q_W (10) = 249.02, $P < 0.001$
Genotyping quality control[c]						
No	255	1	−1.88	−3.04	−0.73	$F_{(1,10)} = 6.54$, $P = 0.028$
Yes	6948	11	−0.50	−0.86	−0.15	$R^2 = 0.37$ Q_W (10) = 145.34, $P < 0.001$

N = total sample size; k = number of studies; d_+ = average effect size; d_L and d_U = lower and upper confidence limits for d_+; F = F-statistic for testing the significance of the moderator; R^2 = proportion of variance accounted for by the moderator; Q_W = statistic for testing the model misspecification. [a]NOS-E3 = no missing data or similar attrition for cases and controls. This last item was not analysed because no study fulfilled it. [b]Controls were assessed for absence of substance use disorder (SUD) with a validated instrument. [c]Reporting of quality control procedures in genotyping methods. CI = confidence interval; ANOVA = analysis of variance.

sizes as the dependent variable. There was no evidence of a relationship between the type of substance and the effect sizes ($F_{(3,7)} = 0.47$, $P = 0.715$, $R^2 = 0$).

Study techniques of telomere length measurement and SUD determination

SUD status was assessed by clinical interview or through self-reported instruments. As shown in Table 5, no relevant differences were found between the two methods of SUD assessment ($P = 0.280$, $R^2 = 0.02$), although the magnitude of the difference in TL between SUD and controls was larger when cases were assessed by clinical interview ($d_+ = -0.73$ versus -0.18). A smaller effect size was found when TL was measured using the quantitative polymerase chain reaction (qPCR) method ($d_+ = -0.55$ versus -0.87), although not reaching statistical significance ($P = 0.482$, $R^2 = 0$). Differences in source tissue used in the biological samples to measure TL did not exhibit a relevant association with the effect sizes ($P = 0.953$, $R^2 = 0$).

Analysis of additional moderating variables

Subgroup analyses [analyses of variance (ANOVA)] were conducted to investigate the potential relationships between clinical, socio-demographic and contextual characteristics and the effect sizes (Table 5). Neither the presence of psychiatric comorbidity ($P = 0.415$, $R^2 = 0$), medical comorbidity ($P = 0.660$, $R^2 = 0$), childhood trauma ($P = 0.771$, $R^2 = 0$) nor exposure to other stressful events ($P = 0.917$, $R^2 = 0$) exhibited a relevant relationship with the effect sizes. Ethnicity of the sample ($P = 0.788$, $R^2 = 0$), country of residence ($P = 0.114$,

Table 5 Subgroup analyses for different characteristics on the effect sizes

Moderator variables	*N*	*k*	d_+	*95% CI* d_L	d_U	*ANOVA results*
Substantive variables						
Type of substance						
Alcohol	4092	6	−0.68	−1.28	−0.08	$F_{(2,8)} = 0.25$, $P = 0.788$
Other	1182	4	−0.86	−1.62	−0.10	$R^2 = 0$ Q_W (8) = 235.87, $P < 0.001$
Alcohol + cocaine	49	1	−0.35	−1.93	1.22	
SUD measurement:						
Clinical interview	4614	10	−0.73	−1.18	−0.27	$F_{(1,10)} = 1.30$, $P = 0.280$
Self-report	2589	2	−0.18	−1.14	0.78	$R^2 = 0.02$ Q_W (10) = 213.34, $P < 0.001$
Telomere measurement:						
qPCR	5225	9	−0.55	−1.04	−0.06	$F_{(1,10)} = 0.53$, $P = 0.482$
Other[a]	1978	3	−0.87	−1.72	0.02	$R^2 = 0$ Q_W (10) = 250.87, $P < 0.001$
Source tissue						
Leucocytes	6268	8	−0.59	−1.15	−0.03	$F_{(2,9)} = 0.05$, $P = 0.953$
Other blood samples	885	3	−0.73	−1.67	0.20	$R^2 = 0$ Q_W (9) = 242.02, $P < 0.001$
Other tissue[b]	50	1	−0.69	−2.37	0.99	
Psychiatric comorbidity?						
Not reported	3432	6	−0.66	−1.26	−0.07	$F_{(2,9)} = 0.97$, $P = 0.415$
Excluded	1182	4	−0.86	−1.60	−0.12	$R^2 = 0$ Q_W (9) = 250.86, $P < 0.001$
Assessed but not excluded	2589	2	−0.09	−1.11	0.91	
Physical comorbidity?						
Not reported	1215	3	−0.62	−1.51	0.26	$F_{(2,9)} = 0.43$, $P = 0.660$
Excluded	1232	5	−0.83	−1.53	−0.13	$R^2 = 0$ Q_W (9) = 194.84, $P < 0.001$
Assessed but not excluded	4756	4	−0.40	−1.16	0.35	
Child trauma						
Not reported	6318	9	−0.60	−1.09	−0.10	$F_{(1,10)} = 0.09$, $P = 0.771$
Assessed but not excluded	885	3	−0.73	−1.60	0.14	$R^2 = 0$ Q_W (10) = 243.98, $P < 0.001$
Other stressful exposures?						
Not reported	5121	9	−0.64	−1.15	−0.13	$F_{(1,10)} = 0.01$, $P = 0.917$
Assessed but not excluded	2082	3	−0.59	−1.44	0.25	$R^2 = 0$ Q_W (10) = 229.07, $P < 0.001$
Contextual variables						
Ethnicity						
Caucasian	2337	2	−0.34	−1.42	0.74	$F_{(2,8)} = 0.25$, $P = 0.788$
Asian	1221	3	−0.96	−1.87	−0.06	$R^2 = 0$ Q_W (8) = 235.87, $P < 0.001$
Arabic	90	1	−0.23	−1.00	0.54	
Mixed	3379	4	−1.48	−3.10	0.14	
Country						
Brazil	176	2	−0.87	−1.74	−0.002	$F_{(6,5)} = 3.15$, $P = 0.114$
China	916	1	−0.31	−1.35	0.73	$R^2 = 0.61$ Q_W (5) = 42.50, $P < 0.001$
Egypt	90	1	−1.48	−2.70	−0.26	
Finland	1880	1	0.32	−0.78	1.43	
Italy	457	1	−0.98	−2.04	0.08	
Japan	305	2	−1.38	−2.21	−0.54	
USA	3379	4	−0.22	−0.77	0.37	
Continent						
Africa	90	1	−1.48	−2.98	0.02	$F_{(4,7)} = 1.30$, $P = 0.357$
Asia	1221	3	−0.96	−1.80	−0.13	$R^2 = 0.09$ Q_W (7) = 168.64, $P < 0.001$
Europe	2337	2	−0.34	−1.34	0.66	
North America	3379	4	−0.23	−0.94	0.48	
South America	176	2	−0.86	−1.93	0.20	
Funding?						
Yes	7113	11	−0.55	−0.97	−0.14	$F_{(1,10)} = 1.91$, $P = 0.196$
Unclear	90	1	−1.48	−2.91	−0.005	$R^2 = 0.08$ Q_W (10) = 234.82, $P < 0.001$

N = total sample size; k = number of studies; d_+ = average effect size; d_L and d_U = lower and upper confidence limits for d_+; F = F-statistic for testing the significance of the moderator; R^2 = proportion of variance accounted for by the moderator. Q_W = statistic for testing the model misspecification; ANOVA = analysis of variance; qPCR = quantitative polymerase chain reaction. [a]'Q-FISH' (quantitative fluorescence *in-situ* hybridization), 'TAGGG telomere length assay kit' and 'Southern blot analysis of terminal restriction fragment lengths'. [b]'Oesophageal mucosa'.

$R^2 = 0.61$) or continent ($P = 0.357$, $R^2 = 0.09$) where the study was conducted or funding type ($P = 0.196$, $R^2 = 0.08$) did not seem to affect the TL–SUD association.

Meta-regressions were applied to assess the influence of unbalanced distribution of several socio-demographic moderators on the TL–SUD association. As shown in Table 6, none of them reached a relevant association with the effect sizes: mean age and SD of the samples (total, cases and controls), percentage of males or study publication year, all of them exhibiting percentages of variance accounting for lower than 10%. However, the total sample size of the studies exhibited a strong relationship with the effect sizes, with 54% of variance accounted for ($P = 0.007$; $R^2 = 0.54$; see Table 6). Supporting information, Fig. S1 presents a scatter-plot of how sample size affected the TL–SUD association. In particular, studies with small sample sizes exhibited stronger TL–SUD associations than studies with larger sample sizes. In other words, studies with small sample sizes found that SUD samples presented shortened TL in a larger magnitude than studies with large sample sizes. This result was coherent with the result of the Egger test described above.

DISCUSSION

To the best of our knowledge, this systematic review and meta-analysis is the first to systematically assess the TL–SUD association. The main result of a total of 12 analysis units suggests that people diagnosed with a SUD have a shorter TL compared to controls. This finding is consistent with other recent meta-analyses suggesting that a shorter TL is associated with (i) other mental disorders [19] such as depression [13,14], post-traumatic stress disorder [17], anxiety [62] and schizophrenia [15,18]; (ii) cigarette smoking [29] and (ii) with other chronic age-related diseases, such as metabolic syndrome [7], diabetes mellitus [8], hypertension [9], cardiometabolic outcomes [10] and cardiovascular disease [11] and Alzheimer's disease [12].

Several strengths of our study should be highlighted. First, data on several potential moderating factors (e.g. childhood adversities, exposure to other stressful events and psychiatric and physical comorbidities) was evaluated. Secondly, quality assessment was implemented [63] using the NOS [33] and, although a TQS was calculated, each item was assessed individually in their influence on the magnitude of the effect [32]. Thirdly, we have evaluated risk of bias [63] and applied GRADE criteria to assess the quality of evidence [50]. Finally, we have used the PRISMA [31] and MOOSE checklists when writing this report [32]; the protocol was registered in PROSPERO and has recently been published [30].

Nevertheless, some limitations deserve careful consideration. At the study level these were: first, some difficulties to extract some characteristics from the studies due to incomplete reporting and a very low quality of evidence based on GRADE criteria [50]. The Strengthening the

Table 6 Results of the mixed-effects meta-regressions for continuous moderators on the effect sizes

	k	*Min.*	*Max.*	*Mean*	b_j	*t*	*P*	Q_E	*P*	R^2
Substantive variables										
Year	12	2011	2019	2016	−0.014	−0.23	0.821	237.68	< 0.001	0
Total mean age	12	34.2	74.8	50.5	0.013	1.02	0.330	197.38	< 0.001	0.004
Case mean age	11	26.2	74.5	47.4	0.010	0.81	0.436	176.60	< 0.001	0
Control mean age	12	33.3	75.1	55.7	0.006	0.48	0.643	213.38	< 0.001	0
Total SD of age	10	2.9	21.9	9.6	−0.040	−0.97	0.358	189.80	< 0.001	0.006
Case SD of age	9	0.7	10.8	6.9	−0.046	−0.56	0.593	193.22	< 0.001	0
Control SD of age	10	0.6	11.2	6.7	−0.079	−1.12	0.297	207.12	< 0.001	0.01
Total percentage male	11	0	100	61.5	−0.006	−1.06	0.315	184.03	< 0.001	0.03
Case percentage male	11	0	100	66.1	−0.004	−0.84	0.425	190.74	< 0.001	0
Control percentage male	12	0	100	56.1	−0.007	−1.34	0.210	186.57	< 0.001	0.08
Methodological variables										
Total sample size	**12**	**49**	**1880**	**600**	**0.0007**	**3.34**	**0.007**	**105.28**	**< 0.001**	**0.54**
Mean age difference[a]	11	−42.1	10.7	−7.7	0.005	0.45	0.662	230.31	< 0.001	0
SD of age difference[b]	9	−1.1	1.04	−0.2	0.094	0.23	0.822	186.30	< 0.001	0
Percentage male difference[b]	11	0	50	9.0	0.012	0.91	0.384	200.70	< 0.001	0
NOS total score[c]	12	1	6	4.6	0.161	1.26	0.237	230.42	< 0.001	0.07

k = number of studies; min. and max. = minimum and maximum values of the moderator variable; b_j = regression coefficient of the moderator; *t* = statistic for testing the significance of the moderator; Q_E = statistic for testing the model misspecification; R^2 = proportion of variance accounted for by the moderator. Bold type highlights the moderator that reached statistical significance. [b]Standard deviation (SD) of age difference = age SD of cases minus age SD of controls. [a]Mean age difference = mean age of cases minus mean age of controls. [b]Percentage male difference = percentage of male of cases minus percentage of males in controls. [c]Range of NOS total score: 0–9.

REporting of Genetic Association studies (STREGA) Statement was published in 2009 [64] as an extension of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) [65] and was specifically designed to enhance the transparency of the reports of genetic association studies based on observational designs. While all 10 studies were published afterwards, none of them has followed these international recommendations. Secondly, the scarce number of included studies limited the ability to identify potential moderators of the association. In our attempts to explain the large heterogeneity observed, only two methodological characteristics were identified as moderators of the TL–SUD association. However, other factors have been previously described (e.g. childhood adversities [66], exposure to other stressful events [67], cigarette smoking [29], physical [7–12] and psychiatric comorbidities [13–19]). Moreover, concerns about the impact of different measurement techniques and variability in several critical methodological steps in measuring TL which may vary between cases and controls, such as sample type selection, protocol of sample collection, storage, processing issues, the lapse of time between sample collection and analyses and assay procedures, among others, have been recently published [68–70]. As a consequence, in an effort to improve the quality of telomere length research, a checklist of the minimum critical information necessary to enhance reproducibility between laboratories, reliability and methodological rigor has been proposed [70]. Thirdly, small study effect is suggested by our analyses, such that the most precise studies (i.e. with large sample sizes) were those that exhibited a very weak TL–SUD association, whereas studies with small sample sizes were those that obtained the largest TL–SUD associations. Fourthly, all were case–control studies except two studies (with three analysis units) that were cohorts in design but used a nested case–control analysis [53,71] with TL measured at a single point in time. Only one of the latter, the Heart and Soul Study described in [53], measured TL in a prospective manner, although the median absolute change in TL was not significant between alcohol consumers and abstainers after 5-year follow-up.

At the review level, the analyses were based on unadjusted effect estimates. Using unadjusted effect estimates in place of adjusted estimates can lead to biased estimates of meta-analytical parameters, such that the results must interpreted with caution. Another limitation was that the scarcity of studies limited subgroup or stratified analyses of individual substances. In addition, the results of the analyses must be interpreted with caution due to the large number of moderating variables analysed and the small number of studies meta-analysed.

Finally, the causal nature of the association between SUDs and TL needs to be interpreted with caution, due to other potential explanations and limitations of current research on this topic. A plausible mechanism is that consumption of illicit drugs might misbalance the equilibrium of telomere addition by telomerase and telomere attrition due to DNA end replication and other factors, e.g. stressful experiences elevating oxidative stress [72,73]. However, this traditional causal explanation of the association of a shorter TL and SUDs has recently been questioned [74]. Telomeres are specialized structures and their complex functionality still needs to be clearly understood, as they cannot be considered as a passive marker of ageing, but also as essential for genome stability and its protection as well as implicated in its expression [1,2].

Future research should improve several aspects in designing and reporting studies (e.g. state in the methods sections that the TL measurements were assessed blind to the condition of participants and to warrant that controls pertain to the same population than cases). Longitudinal studies are needed to establish a temporal relationship between TL and SUDs and to contribute to the clarification of the nature and direction of the relationship. High-quality prospective studies with larger samples will contribute to ascertain the complex nature of the relationship between shortened TL in SUDs. Finally, relevant statistical information is very frequently missing in the studies; in particular, adjusted means and SDs. Studies should report adjusted effect estimates to improve the interpretability of their results.

In summary, we have demonstrated that a shortened TL is associated to SUDs. Although noteworthy, caution should be kept in mind when interpreting these results, as several methodological issues may alternatively explain these findings. If confirmed, TL is a promising marker of accelerated biological ageing in people with SUDs, a potential biomarker for prevention of premature morbidity and mortality and as a viable predictor of different pharmacological [75–77] and non-pharmacological [78,79] interventions.

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Data access statement

All data generated and data sets used during the current study will be available from the corresponding author on reasonable request.

Declaration of interests

F.N.M. reports non-financial support from Otsuka outside the submitted work. The others coauthors declare no conflict of interests.

Author contributions

Fernando Navarro-Mateu: Conceptualization; data curation; methodology; project administration; supervision; validation; writing-original draft; writing-review & editing. **Mathilde Husky:** Conceptualization; writing-original draft; writing-review & editing. **Pedro Cayuela-Fuentes:** Data curation; investigation; writing-review & editing. **Francisco-Javier Álvarez:** Data curation; investigation; writing-review & editing. **Agustín Roca-Vega:** Investigation; resources; writing-review & editing. **María Rubio-Aparicio:** Formal analysis; investigation; methodology; writing-review & editing. **M Dolores Chirlaque:** Conceptualization; investigation; supervision; writing-review & editing. **María Luisa Cayuela:** Conceptualization; methodology; supervision; writing-review & editing. **Salvador Martínez:** Conceptualization; supervision; writing-review & editing. **Julio Sánchez-Meca:** Conceptualization; formal analysis; investigation; methodology; supervision; writing-original draft; writing-review & editing.

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Supporting Information

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

Table S1 Search strategy by electronic database: MEDLINE, EMBASE, PsychInfo and Web of Science (WOS) up to December, 2019.
Table S2 Data extraction for calculating the standardized mean differences and adjusted statistical analyses reported.
Table S3 Excluded studies (alphabetic order)
Table S4 Grading of Recommendations Assessment, Development and Evaluation (GRADE) assessment
Figure S1 Scatter plot of the relationship between sample size and effect size.