Journal of Psychiatric Research 111 (2019) 83–88

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

Telomere shortening in blood leukocytes of patients with posttraumatic stress disorder

Diana Avetyan[a,b,*], Roksana Zakharyan[a,c], Martin Petrek[d], Arsen Arakelyan[a,c]

[a] Laboratory of Human Genomics and Immunomics, Institute of Molecular Biology National Academy of Sciences RA, Yerevan, Armenia
[b] Department of Pathological Physiology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic
[c] Institute of Biomedicine and Pharmacy, Russian-Armenian University, Yerevan, Armenia
[d] Laboratory of Immunogenomics, Department of Pathological Physiology, Faculty of Medicine and Dentistry, Institute of Molecular and Translational Medicine, Palacky University Olomouc, Olomouc, Czech Republic

ARTICLE INFO

Keywords:
Posttraumatic stress disorder
PTSD
Relative leukocyte telomere length
Telomerase
TERC
TERT
SNPs
qPCR
PCR-SSP

ABSTRACT

Telomeres are protective fragments on chromosome ends involved in maintaining genome stability, preventing chromosomal fusions, regulation of cell division. It was shown that telomere attrition rate is accelerated in age-related diseases, as well as in response to physiological and psychosocial stress. The aim of this study was to evaluate relative leukocyte telomere length (LTL) in patients with post traumatic stress disorder (PTSD), as well as to investigate association of functional SNPs of telomerase *TERC* and *TERT* genes with LTL and PTSD. The relative LTL was measured by multiplex quantitative PCR method; genotyping of *TERC* rs12696304, *TERT* rs7726159 and rs2736100 was performed by PCR with sequence specific primers. Comparison of LTL in diseased and healthy subjects showed that PTSD patients had shorter average LTL than controls. Also, the frequency and the carriage rate of the *TERT* rs2736100*T allele was higher in PTSD patients compared to controls. Overall our results are in line with previous research in different populations. Furthermore, we have demonstrated that rs2736100 of *TERT* gene was significantly associated with PTSD and the minor allele of this polymorphism may be considered as a risk factor for PTSD in the Armenian population.

1. Introduction

Posttraumatic stress disorder (PTSD, ICD-10 codes: F43.1; DSM-V code: 309.81) is a severe mental disorder and is characterized by polygenic nature and poorly determined pathogenesis (Logue et al., 2015). It is commonly accepted that the development of this disease is a result of complex interplay between environmental and genetic factors (American Psychiatric Association, 2013; World Health Organisation, 2016).

Telomeres refer to protective non-coding complexes of tandem TTAGGG repeats at the ends of chromosomes and are involved in maintaining genomic stability, regulation of gene expression and prevention of chromosomal fusion (Chan and Blackburn, 2004) (Weinrich et al., 1997). They may shorten during repeated cell divisions, aging, and in response to several environmental factors, such as psychological (Epel et al., 2004) and physiological stress (Lindqvist et al., 2015), smoking, obesity, and high parental age (Malaspina et al., 2014; Morla et al., 2006). Telomere length therefore has been suggested to be a biomarker of cellular aging, age-related diseases, as well as psychosocial stress.

Numerous studies indicated that telomere length (TL) and attrition rate correlate with poorer health, and predict mortality in certain psychiatric disorders (Lindqvist et al., 2015; Polho et al., 2015; Simon et al., 2006). Moreover, it was also shown that TL correlates with cognitive ability in healthy individuals (Valdes et al., 2010; Yaffe et al., 2011). The latest studies have shown that in parallel with established biochemical and physiological stressors, mental traumatic stress was also associated with accelerated shortening of telomeres (Codd et al., 2010; Damjanovic et al., 2007; Epel et al., 2004).

Telomere length in healthy cells is highly regulated in a tissue- and cell type-specific manner and is dependent on mitotic turnover rate, telomerase activity, and telomerase-associated factors. Telomerase is a ribonucleoprotein polymerase, that consists of a telomerase catalytic component with reverse transcriptase activity (TERT) and a telomerase RNA component (TERC), serving as a template for telomere repeat synthesis (Weinrich et al., 1997). In healthy human organism, telomerase is highly active in germline and stem cells and exhibits mild activity in leukocytes (Bekaert et al., 2004; Hiyama and Hiyama, 2007;

* Corresponding author. Laboratory of Human Genomics and Immunomics, Institute of Molecular Biology National Academy of Sciences RA, Yerevan, Armenia.
E-mail address: d_avetyan@mb.sci.am (D. Avetyan).

https://doi.org/10.1016/j.jpsychires.2019.01.018
Received 6 November 2018; Received in revised form 14 January 2019; Accepted 21 January 2019

Table 1
Age characteristics of studied groups.

Variables	Controls	PTSD	P-value
N (male)	49	41	
Age, years (M ± m)	43.5 ± 9.4	46.4 ± 7.63	0.098
Max. age, years	65	63	
Min. age, years	29	34	

Xu et al., 2017). A number of studies implicated polymorphisms in genes coding for telomerase components in several psychiatric disorders, such as schizophrenia, major depressive disorder, depression, etc. (Michalek et al., 2017; Rao et al., 2016; Wei et al., 2016).

The aim of this study was to evaluate relative leukocyte telomere length (LTL) in patients with PTSD and control group in Armenian population, and also to investigate the association of relative LTL, functional SNPs of the *TERC* and *TERT* genes with PTSD.

2. Methods

2.1. Study population

Study population includes the following groups (Table 1):

- combat veterans with PTSD (n = 41);
- age-, sex-matched, unrelated physically and mentally healthy volunteers (control group) with no family history of mental disorder (n = 49).

Blood samples of PTSD-affected subjects were available from "Stress" Department of Mental Health Rehabilitation, "ArtMed" Medical Center of RA, where the diagnosis was done following the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (SCID-I) and Clinical-administered PTSD Scale (CAPS) (Table 2). Blood samples of healthy subjects (controls) were obtained from blood donors. Exclusion criteria for healthy subjects included any psychiatric disease during lifetime, any serious neurological or endocrine disorder, any medical condition or treatment known to affect the brain, or meeting DSM-IV criteria for mental retardation as determined from the non-patient version of the Structured Clinical Interview for DSM-IV-TR Axis I Disorders. Finally, exclusion criteria for all study participants included any serious medical disorder. Informed consent to participate in this study was obtained from each subject. The study was verified approved by the Ethical Committee (IRB#00004079) of the Institute of Molecular Biology of the National Academy of Sciences (NAS) RA.

Table 2
Clinician-Administered PTSD Scale (CAPS) scoring in PTSD patients.

		Score (M ± m)
Criterion B (Re-experiencing symptoms)	Frequency (0–20)	12.17 ± 0.6
	Intensity (0–20)	11.95 ± 0.6
	Frequency + Intensity (0–40)	24.12 ± 1.05
Criterion C (Avoidance symptoms)	Frequency (0–28)	15.39 ± 0.7
	Intensity (0–28)	14.61 ± 0.8
	Frequency + Intensity (0–56)	30.0 ± 1.8
Criterion D (Arousal symptoms)	Frequency (0–20)	13.07 ± 0.4
	Intensity (0–20)	12.22 ± 0.4
	Frequency + Intensity (0–40)	25.29 ± 0.8
Total criterion (B + C + D)	Frequency (0–68)	39.88 ± 1.6
	Intensity (0–68)	38.05 ± 1.8
	Frequency + Intensity (0–136)	77.93 ± 3.3

Table 3
Primers of the PCR assays for detection of polymorphisms in TERC and TERT loci.

SNP	Nucleotide sequence of primers
rs12696304	standard allele: 5′ - ATC TTA GAT CAC CTT GAG TAA AC minor allele: 5′ - ATC TTA GAT CAC CTT GAG TAA AG constant: 5′ - TGG AAT TGT CTA GCA GAT ACA TT
rs7726159	standard allele: 5′ - CAG GAG TTT GTG CCA AGT GG minor allele: 5′ - CAG GAG TTT GTG CCA AGT GT constant: 5′ - TGA GGC TGG TGA ATC GCT TAA
rs2736100	standard allele: 5′ - TTT CCG TGT TGA GTG TTT CTG minor allele: 5′ - TTT CCG TGT TGA GTG TTT CTT constant: 5′ - CTG TGC ATC ATA AGC AGA GGT

2.2. Blood sampling and genomic DNA extraction

The experiments were performed using genomic DNA samples of study subjects. Genomic DNA was isolated from fresh blood samples according to Miller's salting-out procedure (Miller et al., 1988) modification, where proteinase K was omitted and chloroform extraction phase was added (Bunce et al., 1999). Upon DNA isolation samples were stored at −30 °C until further use.

2.3. Primer design for PCR-SSP

DNA samples were genotyped for *TERC* rs12696304; *TERT* rs7726159 and rs2736100 functional SNPs (Table 3). The SNPs were selected based on previous genome-wide association studies (GWAS) (Codd et al., 2013, 2010; Pooley et al., 2013).

2.4. Polymerase chain reaction with sequence specific primers

Genotyping was carried out by polymerase chain reaction with sequence-specific primers (PCR-SSP) according to protocol published in Bunce et al. (1995). Final reaction volume (25 μl) consisted of 1x Thermo-Start PCR Buffer, 0.2 mM of each dNTP, 0.5 μM forward primer, the same amount of reverse primer, 2 mM of 25 mM $MgCl_2$, 20 ng template DNA, 0.625 U Taq polymerase and nuclease-free water. PCR-SSP was performed as follows: 95 °C for 15 min (initial denaturation), followed by 35 cycles of 95 °C for 20 s (denaturation), 55–60 °C for 30 s (annealing) and 72 °C for 1 min (extension), and final cycle of 72 °C for 5 min (final extension).

The presence/absence of allele-specific amplicons in the PCR products was visualized in 2% agarose gel stained with ethidium bromide fluorescent dye using DNA molecular weight markers as reference. To check the reproducibility of results, randomly selected DNA samples (10% of total) were genotyped twice.

2.5. Measurement of leukocyte telomere length

The relative leukocyte telomere length (LTL) was measured by monochrome multiplex quantitative PCR method, as described by Cawthon with minor modifications (Cawthon, 2009). Briefly, this method describes the relative telomere length as the ratio (T/S) of the telomere repeat copy number (T) to a single copy gene (S). This ratio is measured relative to a standard DNA which in this case was a reference representing pooled DNA sample from five healthy subjects (Cawthon, 2009).

The primers (5′→3′) used for the telomere amplification were following: telg (900 nM): ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT, and telc (900 nM): TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA. The predicted product size was 76 bp. The primers for amplification of the single copy gene (scg) beta-globin were hbgu (500 nM): CGGCGGCGGGCGGCGCGGGCTGGGCGGcttcatccacgttcaccttg and hbgd (500 nM): GCCCGGCCCGCCGCGCCCGTCCCGCCGgaggagaagtctgccgtt. The predicted

product size was 106 bp. Capitalized bases are non-templated 50 tag sequences that confer a very high melting temperature on the resulting PCR product. The thermal cycling profile for the qPCR was as follows: 95 °C for 15 min, followed by 2 cycles of 94 °C for 15 s and 49 °C for 60 s, 4 cycles of 94 °C for 15 s and 59 °C for 30 s, 20 cycles of 85 °C for 15 s and 59 °C for 30 s with fluorescent signal acquisition, 27 cycles of 94 °C for 15 s, 84 °C for 10 s and finally 85 °C for 15 s with fluorescent signal acquisition. The final reaction volume (10 μl) consisted of 1 × SYBR Green PCR Master Mix (Applied Biosystems), 20 ng of template, and the respective primers.

After thermal cycling the mean Ct values for both telomeres and the single copy gene for were used to calculate the relative T/S ratio for each assayed sample. The relative T/S ratio (T/S of one sample relative to T/S of another sample) was determined by the ΔΔCt method, using formula $T/S = 2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct_{telomere} - Ct_{\beta\text{-globin}})$ of the sample $- (Ct_{telomere} - Ct_{\beta\text{-globin}})$ of the reference.

2.6. Statistical analyses

The distributions of genotypes for selected SNPs were checked for correspondence to Hardy-Weinberg equilibrium (HWE). The potential association of genotype, allele frequencies and minor allele carriage rate of selected SNPs with PTSD was evaluated by comparing the significance of differences between study groups in additive, dominant and recessive models using Fisher's exact test. The odds ratio (OR), 95% confidence interval (CI), and exact p value ($p_{nominal}$) were calculated. P values adjusted by Bonferroni multiple comparison correction are further indicated as $p_{corrected}$. $P_{corrected}$ values < 0.05 were considered statistically significant. The Mann-Whitney U test was used to evaluate the possible differences of relative LTL between studied groups, as well as relative LTL between minor allele carrier and non-carrier PTSD patients. Data on relative LTL is presented as mean ± SD. Genotyping data is presented in absolute counts and proportions.

3. Results

The telomere length was measured in 41 patients with PTSD and 49 healthy subjects. The results showed that there is negative relationship between LTL and age in healthy subjects, though not significant. These may be because of a very narrow age range of subjects involved in this study (46 ± 8 years, 53% of them were in range of 40–50). Comparison of LTL in diseased and healthy subjects showed that PTSD patients had 1.5 times shorter average LTL than controls (T/S ratio: 0.91 ± 0.07 vs. 1.33 ± 0.14, p = 0.03, Fig. 1). No significant correlation was observed between CAPS scores and LTL.

Furthermore, the associations between the *TERC* rs12696304, *TERT* rs7726159 and rs2736100 polymorphisms and PTSD risk were calculated based on dominant, recessive and additive models of genetic associations (Table 4). The distribution of genotypes for all selected SNPs in all study groups complied with HWE.

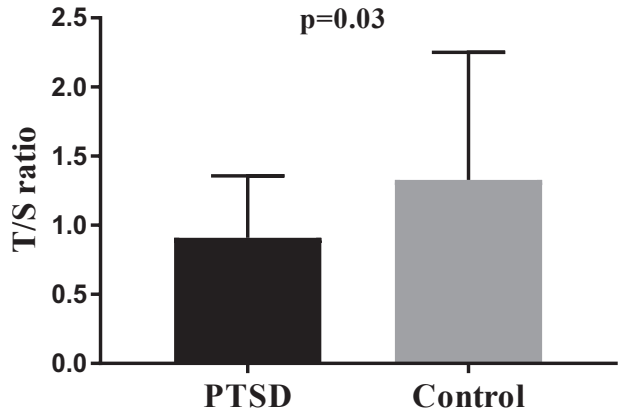

Fig. 1. Relative LTL in PTSD patients and controls.

Table 4
Associations between the TERC rs12696304, TERT rs7726159 and rs2736100 polymorphism and overall PTSD risk.

Gene, SNP		Controls (n = 49)	PTSD (n = 41)	Odds ratio		p value[a]
				OR	95% CI	
***TERC* rs12696304**						
Genotypes	CC	24 (0.49)	16 (0.39)			
	CG	19 (0.39)	19 (0.46)			
	GG	6 (0.12)	6 (0.15)			
Additive model	C	67 (0.68)	51 (0.62)			0.386
	G	31 (0.32)	31 (0.38)	0.761	[0.411–1.411]	
Dominant model	G	25 (0.51)	25 (0.61)	1.5	[0.647–3.478]	0.344
Recessive model	G	6 (0.12)	6 (0.15)	1.23	[0.364–4.146]	0.74
***TERT* rs7726159**						
Genotypes	GG	17 (0.35)	17 (0.41)			
	GT	25 (0.51)	18 (0.44)			
	TT	7 (0.14)	6 (0.15)			
Additive model	G	59 (0.60)	52 (0.63)			0.659
	T	39 (0.40)	30 (0.37)	1.15	[0.626–2.097]	
Dominant model	T	32 (0.65)	24 (0.59)	0.75	[0.319–1.765]	0.510
Recessive model	T	7 (0.14)	6 (0.15)	1.03	[0.316–3.344]	1
***TERT* rs2736100**						
Genotypes	GG	20 (0.41)	8 (0.20)			
	GT	19 (0.39)	14 (0.34)			
	TT	10 (0.20)	19 (0.46)			
Additive model	G	59 (0.60)	30 (0.37)			**1.60E-03**
	T	39 (0.40)	52 (0.63)	0.381	[0.208–0.698]	
Dominant model	T	29 (0.59)	33 (0.81)	2.845	[1.09–7.428]	**0.02969**
Recessive model	T	39 (0.80)	22 (0.54)	0.297	[0.117–0.75]	**0.009**

[a] $p_{nomimal}$ values of significance are given.

According to the results obtained, the frequency (additive model) of the *TERT* rs2736100*T allele was 1.6 times higher in PTSD patients compared to controls (0.63 vs. 0.40, $p_{corrected}$ = 0.005). Also, the carriage rate of the rs2736100*T minor allele (dominant model) were 1.4 times higher in PTSD patients than in control group (0.81 vs. 0.59, $p_{corrected}$ = 0.03).

Considering dual nature of association of PTSD with both relative LTL and *TERT* rs2736100 SNP, we performed multiple logistic regression analysis including relative LTL, *TERT* rs2736100 genotypes as well as age of studied subjects as predictors and PTSD as a response variable. The results showed that difference in *TERT* rs2736100 genotype frequencies and relative LTL between the patient and control groups remained significant (Table 5). No significant difference was observed in CAPS scores between patients with different *TERT* rs2736100 genotypes.

In addition we noticed that the patients carrying the *TERT* rs2736100*T minor allele had the median level of TL 1.3 times lower than in standard allele homozygotes (Fig. 2), however, this difference was not significant (p = 0.3).

Table 5
Multiple logistic regression[a] coefficients for relative LTL, TERT rs2736100 genotypes and age of studied subjects.

	Estimate	Std. Error	t value	Pr(> |t|)
(Intercept)	0.348	0.304	1.144	0.256
TERT rs2736100	0.157	0.067	2.346	0.021
LTL	−0.160	0.069	−2.317	0.023
Age	0.003	0.006	0.485	0.629

[a] Response variable: PTSD status.

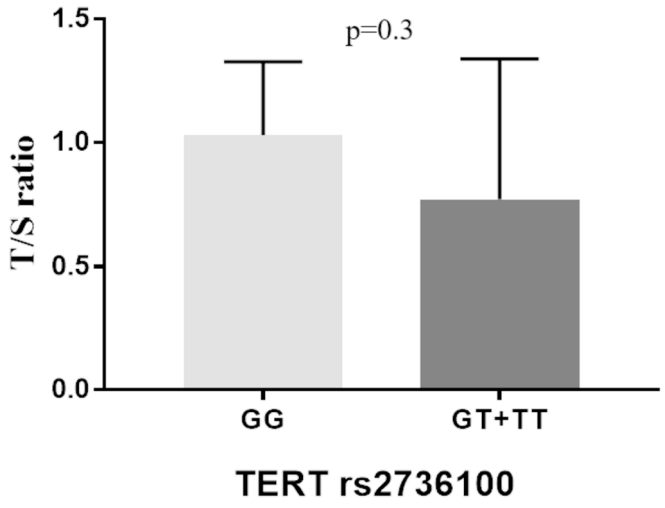

Fig. 2. Relative LTL in PTSD patients depending on rs2736100 genotypes.

Finally, according to our results there was no association found between *TERC* rs12696304 and *TERT* rs7726159 polymorphisms and PTSD or LTL.

4. Discussion

The relationship between telomere length and psychiatric disorders has been a topic of great interest, but also of uncertainty. Data from clinical studies suggest that shorter leukocyte telomere length (LTL) can be associated with many diseases including autoimmune and metabolic diseases, cancer, stroke, cardiovascular disease, diabetes and diseases that are associated with common psychiatric disorders (Bernal and Tusell, 2018; Cawthon, 2009). There is also evidence for decreased telomere length in patients with mood disorders, such as major depressive disorder and bipolar disorder, different forms of dementia, schizophrenia (Kao et al., 2008; Lung et al., 2007; Powell et al., 2018; Simon et al., 2006).

The present study investigated whether leukocyte LTL is influenced by trauma and PTSD in a population of Armenian males exposed to the same traumatic event. Our results suggest that PTSD may accelerate cellular aging as reflected by decreased telomere length. Although the sample size was small, we found a significant 1.5 fold difference between the LTL of PTSD patients compared to controls. Short LTL in PTSD patients were observed regardless the gender (Li et al., 2017), while conflicting data is available on the trauma type that triggers the disease. Thus, in the meta-analysis by sexual assault and childhood trauma but not combat trauma were associated with short LTL (Li et al., 2017). However, while no LTL differences were also observed in PTSD and non-PTSD groups of Korean male veterans, shorter LTL were found after stratification of patients by combat severity (Kim et al., 2017). Bersani et al. reported that early trauma, severity of perceived stress and general psychopathological symptoms were more closely associated with shorter TL than is the severity of core diagnostic symptoms of PTSD (Bersani et al., 2016). Finally, in another study significant age-dependent shortening of telomeres were noted in war veterans with PTSD (Jergović et al., 2014). Furthermore, several studies have shown that stress and increased cortisol levels can lead to the shortening of telomeres in lymphocytes (Choi et al., 2008). Moreover, the link between short LTL and reduced hippocampal volume commonly associated with PTSD was observed previously (Mamdani et al., 2015; Nilsonne et al., 2015).

Thus our results from one side indicate that combat trauma is associated with shortening of telomeres in PTSD as well as support the theory of accelerated aging in the disease (Lohr et al., 2015), as it has been also documented for other psychiatric disorders (Damjanovic et al., 2007; Powell et al., 2018; Simon et al., 2006).

Genome-wide association studies (GWAS) have shown that LTL is influenced by a number of genetic variants. Several loci have been shown to be associated with LTL, including: TERC (3p26), TERT (5p15.33), *ACYP2* (2p16.2), NAF1 (10q24.33), OBFC1 (10q24.33), DCAF4 (14q24.2), CTC1 (17p13.1), ZNF208 (19p12), ZNF676 (19p12) and RTEL1 (20q13.3) (Codd et al., 2013; Pooley et al., 2013; Weng et al., 2016). Among them, common variants of *TERC* and *TERT* show repeated association with mean LTL in GWAS studies (Codd et al., 2013, 2010; Pooley et al., 2013). However, to our knowledge, genetic association analysis between *TERC* and *TERT* variants and PTSD has not been studied before.

We have, therefore, analyzed genetic polymorphisms of telomerase components (TERT and TERC) as plausible predictors of leukocyte LTL. Selected TERC and TERT SNPs are located in intronic regions of TERC and TERT, respectively. Our analysis revealed that variation in the rs2736100 of *TERT* gene was associated with PTSD in our patients group. In addition, we noticed overrepresentation of rs2736100*G allele in the Armenian population, while it is reported as minor allele in European population (Kishore et al., 2016). On the other hand, rs2736100*G allele is considered as major allele in South Asian populations (Seow et al., 2017). We also compared genotypes distribution with GWAS data for Armenians from Haber et al. (2016) and found similar allele frequencies as in our control group (MAF = 0.399, N = 99 samples were collected from Eastern Armenia). According to our knowledge, this is the first study showing association between rs2736100 TERT variant with PTSD and the minor allele (T) of rs2736100 may be considered as a risk factor for PTSD. This finding should, however, be replicated in another Armenian cohort, and also in other populations.

The data on shortened LTL presented in this study is in line with previous research in different populations and extend the current knowledge by reporting association between PTSD and genetic variation in telomerase TERT component reported for the first time. A limitation of this study can be considered a small sample size. The strength of our study is that all PTSD patients were males exposed to the same traumatic event in relatively homogenous population.

5. Conclusions

The present study shows that patients with combat PTSD in the Armenian population have shorter telomere length than healthy controls; a finding that is in line with previous research in different populations. Furthermore, this is the first study that showed that rs2736100 of *TERT* gene was significantly associated with PTSD and the minor allele of rs2736100 may be considered as a risk factor for PTSD in the Armenian population.

Declarations of interest

None.

Acknowledgements

This study was supported by Visegrad Fund V4EAP Fellowship to VF (#51700954) and in part by Palacky University (IGA PU LF 2017_014/2018_015). The funding sources had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

References

American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders, 5th ed. American Psychiatric Publishing, Arlington, VA.

Bekaert, S., Derradji, H., Baatout, S., 2004. Telomere biology in mammalian germ cells and during development. Dev. Biol. 274, 15–30. https://doi.org/10.1016/j.ydbio.

2004.06.023.
Bernal, A., Tusell, L., 2018. Telomeres: implications for cancer development. Int. J. Mol. Sci. 19, 294. https://doi.org/10.3390/ijms19010294.
Bersani, F.S., Lindqvist, D., Mellon, S.H., Epel, E.S., Yehuda, R., Flory, J., Henn-Hasse, C., Bierer, L.M., Makotkine, I., Abu-Amara, D., Coy, M., Reus, V.I., Lin, J., Blackburn, E.H., Marmar, C., Wolkowitz, O.M., 2016. Association of dimensional psychological health measures with telomere length in male war veterans. J. Affect. Disord. 190, 537–542. https://doi.org/10.1016/j.jad.2015.10.037.
Bunce, M., O'Neill, C.M., Barnardo, M.C., Krausa, P., Browning, M.J., Morris, P.J., Welsh, K.I., 1995. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). Tissue Antigens 46, 355–367.
Bunce, M., Procter, J., Welsh, K.I., 1999. A DNA-based detection and screening system for identifying HLA class I expression variants by sequence-specific primers. Tissue Antigens 53, 498–506.
Cawthon, R.M., 2009. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res. 37, e21. https://doi.org/10.1093/nar/gkn1027.
Chan, S.R.W.L., Blackburn, E.H., 2004. Telomeres and telomerase. Philos. Trans. R. Soc. Lond. B Biol. Sci. 359, 109–121. https://doi.org/10.1098/rstb.2003.1370.
Choi, J., Fauce, S.R., Effros, R.B., 2008. Reduced telomerase activity in human T lymphocytes exposed to cortisol. Brain Behav. Immun. 22, 600–605. https://doi.org/10.1016/j.bbi.2007.12.004.
Codd, V., Mangino, M., van der Harst, P., Braund, P.S., Kaiser, M., Beveridge, A.J., Rafelt, S., Moore, J., Nelson, C., Soranzo, N., Zhai, G., Valdes, A.M., Blackburn, H., Leach, I.M., de Boer, R.A., Kimura, M., Aviv, A., Goodall, A.H., Ouwehand, W., van Veldhuisen, D.J., van Gilst, W.H., Navis, G., Burton, P.R., Tobin, M.D., Hall, A.S., Thompson, J.R., Spector, T., Samani, N.J., Samani, N.J., 2010. Common variants near TERC are associated with mean telomere length. Nat. Genet. 42, 197–199. https://doi.org/10.1038/ng.532.
Codd, V., Nelson, C.P., Albrecht, E., Mangino, M., Deelen, J., Buxton, J.L., Hottenga, J.J., Fischer, K., Esko, T., Surakka, I., Broer, L., Nyholt, D.R., Leach, I.M., Salo, P., Hägg, S., Matthews, M.K., Palmen, J., Norata, G.D., O'Reilly, P.F., Saleheen, D., Amin, N., Balmforth, A.J., Beekman, M., de Boer, R.A., Böhringer, S., Braund, P.S., Burton, P.R., Craen, A.J.M., Denniff, M., Dong, Y., Douroudis, K., Dubinina, E., Eriksson, J.G., Garlaschelli, K., Guo, D., Hartikainen, A.-L., Henders, A.K., Houwing-Duistermaat, J.J., Kananen, L., Karssen, L.C., Kettunen, J., Klopp, N., Lagou, V., van Leeuwen, E.M., Madden, P.A., Mägi, R., Magnusson, P.K.E., Männistö, S., McCarthy, M.I., Medland, S.E., Mihailov, E., Montgomery, G.W., Oostra, B.A., Palotie, A., Peters, A., Pollard, H., Pouta, A., Prokopenko, I., Ripatti, S., Salomaa, V., Suchiman, H.E.D., Valdes, A.M., Verweij, N., Viñuela, A., Wang, X., Wichmann, H.-E., Widen, E., Willemsen, G., Wright, M.J., Xia, K., Xiao, X., van Veldhuisen, D.J., Catapano, A.L., Tobin, M.D., Hall, A.S., Blakemore, A.I.F., van Gilst, W.H., Zhu, H., Erdmann, J., Reilly, M.P., Kathiresan, S., Schunkert, H., Talmud, P.J., Pedersen, N.L., Perola, M., Ouwehand, W., Kaprio, J., Martin, N.G., van Duijn, C.M., Hovatta, I., Gieger, C., Metspalu, A., Boomsma, D.I., Jarvelin, M.-R., Slagboom, P.E., Thompson, J.R., Spector, T.D., van der Harst, P., Samani, N.J., Samani, N.J., 2013. Identification of seven loci affecting mean telomere length and their association with disease. Nat. Genet. 45, 422–427. https://doi.org/10.1038/ng.2528.
Damjanovic, A.K., Yang, Y., Glaser, R., Kiecolt-Glaser, J.K., Nguyen, H., Laskowski, B., Zou, Y., Beversdorf, D.Q., Weng, N., 2007. Accelerated telomere erosion is associated with a declining immune function of caregivers of Alzheimer's disease patients. J. Immunol. 179, 4249–4254.
Epel, E.S., Blackburn, E.H., Lin, J., Dhabhar, F.S., Adler, N.E., Morrow, J.D., Cawthon, R.M., 2004. Accelerated telomere shortening in response to life stress. Proc. Natl. Acad. Sci. Unit. States Am. 101, 17312–17315. https://doi.org/10.1073/pnas.0407162101.
Haber, M., Mezzavilla, M., Xue, Y., Comas, D., Gasparini, P., Zalloua, P., Tyler-Smith, C., 2016. Genetic evidence for an origin of the Armenians from Bronze Age mixing of multiple populations. Eur. J. Hum. Genet. 24, 931–936. https://doi.org/10.1038/ejhg.2015.206.
Hiyama, E., Hiyama, K., 2007. Telomere and telomerase in stem cells. Br. J. Canc. 96, 1020–1024. https://doi.org/10.1038/sj.bjc.6603671.
Jergović, M., Tomičević, M., Vidović, A., Bendelja, K., Savić, A., Vojvoda, V., Rac, D., Lovrić-Čavar, D., Rabatić, S., Jovanovic, T., Sabioncello, A., 2014. Telomere shortening and immune activity in war veterans with posttraumatic stress disorder. Prog. Neuro Psychopharmacol. Biol. Psychiatr. 54, 275–283. https://doi.org/10.1016/j.pnpbp.2014.06.010.
Kao, H.-T., Cawthon, R.M., Delisi, L.E., Bertisch, H.C., Ji, F., Gordon, D., Li, P., Benedict, M.M., Greenberg, W.M., Porton, B., 2008. Rapid telomere erosion in schizophrenia. Mol. Psychiatr. 13, 118–119. https://doi.org/10.1038/sj.mp.4002105.
Kim, T.Y., Kim, S.J., Choi, J.R., Lee, S.-T., Kim, J., Hwang, I.S., Chung, H.G., Choi, J.H., Kim, H.W., Kim, S.H., Kang, J.I., 2017. The effect of trauma and PTSD on telomere length: an exploratory study in people exposed to combat trauma. Sci. Rep. 7, 4375. https://doi.org/10.1038/s41598-017-04682-w.
Kishore, A., Žižková, V., Kocourková, L., Petrkova, J., Bouros, E., Nunes, H., Loštáková, V., Müller-Quernheim, J., Zissel, G., Kolek, V., Bouros, D., Valeyre, D., Petrek, M., 2016. Association study for 26 candidate loci in idiopathic pulmonary fibrosis patients from four european populations. Front. Immunol. 7, 274. https://doi.org/10.3389/fimmu.2016.00274.
Li, X., Wang, J., Zhou, J., Huang, P., Li, J., 2017. The association between post-traumatic stress disorder and shorter telomere length: a systematic review and meta-analysis. J. Affect. Disord. 218, 322–326. https://doi.org/10.1016/j.jad.2017.03.048.
Lindqvist, D., Epel, E.S., Mellon, S.H., Penninx, B.W., Révész, D., Verhoeven, J.E., Reus, V.I., Lin, J., Mahan, L., Hough, C.M., Rosser, R., Bersani, F.S., Blackburn, E.H., Wolkowitz, O.M., 2015. Psychiatric disorders and leukocyte telomere length: underlying mechanisms linking mental illness with cellular aging. Neurosci. Biobehav. Rev. 55, 333–364. https://doi.org/10.1016/j.neubiorev.2015.05.007.
Logue, M.W., Amstadter, A.B., Baker, D.G., Duncan, L., Koenen, K.C., Liberzon, I., Miller, M.W., Morey, R.A., Nievergelt, C.M., Ressler, K.J., Smith, A.K., Smoller, J.W., Stein, M.B., Sumner, J.A., Uddin, M., 2015. The psychiatric genomics consortium post-traumatic stress disorder workgroup: posttraumatic stress disorder enters the age of large-scale genomic collaboration. Neuropsychopharmacology 40, 2287–2297. https://doi.org/10.1038/npp.2015.118.
Lohr, J.B., Palmer, B.W., Eidt, C.A., Aailaboyina, S., Mausbach, B.T., Wolkowitz, O.M., Thorp, S.R., Jeste, D.V., 2015. Is post-traumatic stress disorder associated with premature senescence? A review of the literature. Am. J. Geriatr. Psychiatry 23, 709–725. https://doi.org/10.1016/j.jagp.2015.04.001.
Lung, F.-W., Chen, N.C., Shu, B.-C., 2007. Genetic pathway of major depressive disorder in shortening telomeric length. Psychiatr. Genet. 17, 195–199. https://doi.org/10.1097/YPG.0b013e32808374f6.
Malaspina, D., Dracxler, R., Walsh-Messinger, J., Harlap, S., Goetz, R.R., Keefe, D., Perrin, M.C., 2014. Telomere length, family history, and paternal age in schizophrenia. Mol. Genet. genomic Med. 2, 326–331. https://doi.org/10.1002/mgg3.71.
Mamdani, F., Rollins, B., Morgan, L., Myers, R.M., Barchas, J.D., Schatzberg, A.F., Watson, S.J., Akil, H., Potkin, S.G., Bunney, W.E., Vawter, M.P., Sequeira, P.A., 2015. Variable telomere length across post-mortem human brain regions and specific reduction in the hippocampus of major depressive disorder. Transl. Psychiatry 5 e636–e636. https://doi.org/10.1038/tp.2015.134.
Michalek, J.E., Kepa, A., Vincent, J., Frissa, S., Goodwin, L., Hotopf, M., Hatch, S.L., Breen, G., Powell, T.R., 2017. Genetic predisposition to advanced biological ageing increases risk for childhood-onset recurrent major depressive disorder in a large UK sample. J. Affect. Disord. 213, 207–213. https://doi.org/10.1016/j.jad.2017.01.017.
Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.
Morla, M., Busquets, X., Pons, J., Sauleda, J., MacNee, W., Agustí, A.G.N., 2006. Telomere shortening in smokers with and without COPD. Eur. Respir. J. 27, 525–528. https://doi.org/10.1183/09031936.06.00087005.
Nilsonne, G., Tamm, S., Månsson, K.N.T., Åkerstedt, T., Lekander, M., 2015. Leukocyte telomere length and hippocampus volume: a meta-analysis. F1000Research 4, 1073. https://doi.org/10.12688/f1000research.7198.1.
Polho, G.B., De-Paula, V.J., Cardillo, G., dos Santos, B., Kerr, D.S., 2015. Leukocyte telomere length in patients with schizophrenia: a meta-analysis. Schizophr. Res. 165, 195–200. https://doi.org/10.1016/j.schres.2015.04.025.
Pooley, K.A., Bojesen, S.E., Weischer, M., Nielsen, S.F., Thompson, D., Amin Al Olama, A., Michailidou, K., Tyrer, J.P., Benlloch, S., Brown, J., Audley, T., Luben, R., Khaw, K.-T., Neal, D.E., Hamdy, F.C., Donovan, J.L., Kote-Jarai, Z., Baynes, C., Shah, M., Bolla, M.K., Wang, Q., Dennis, J., Dicks, E., Yang, R., Rudolph, A., Schildkraut, J., Chang-Claude, J., Burwinkel, B., Chenevix-Trench, G., Pharoah, P.D.P., Berchuck, A., Eeles, R.A., Easton, D.F., Dunning, A.M., Nordestgaard, B.G., 2013. A genome-wide association scan (GWAS) for mean telomere length within the COGS project: identified loci show little association with hormone-related cancer risk. Hum. Mol. Genet. 22, 5056–5064. https://doi.org/10.1093/hmg/ddt355.
Powell, T.R., Dima, D., Frangou, S., Breen, G., 2018. Telomere length and bipolar disorder. Neuropsychopharmacology 43, 445–453. https://doi.org/10.1038/npp.2017.125.
Rao, S., Ye, N., Hu, H., Shen, Y., Xu, Q., 2016. Variants in TERT influencing telomere length are associated with paranoid schizophrenia risk. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 171B, 317–324. https://doi.org/10.1002/ajmg.b.32403.
Seow, W.J., Matsuo, K., Hsiung, C.A., Shiraishi, K., Song, M., Kim, H.N., Wong, M.P., Hong, Y.-C., Hosgood, H.D., Wang, Z., Chang, I.-S., Wang, J.-C., Chatterjee, N., Tucker, M., Wei, H., Mitsudomi, T., Zheng, W., Kim, J.H., Zhou, B., Caporaso, N.E., Albanes, D., Shin, M.-H., Chung, L.P., An, S.-J., Wang, P., Zheng, H., Yatabe, Y., Zhang, X.-C., Kim, Y.T., Shu, X.-O., Kim, Y.-C., Bassig, B.A., Chang, J., Ho, J.C.M., Ji, B.-T., Kubo, M., Daigo, Y., Ito, H., Momozawa, Y., Ashikawa, K., Kamatani, Y., Honda, T., Sakamoto, H., Kunitoh, H., Tsuta, K., Watanabe, S.-I., Nokihara, H., Miyagi, Y., Nakayama, H., Matsumoto, S., Tsuboi, M., Goto, K., Yin, Z., Shi, J., Takahashi, A., Goto, A., Minamiya, Y., Shimizu, K., Tanaka, K., Wu, T., Wei, F., Wong, J.Y.Y., Matsuda, F., Su, J., Kim, Y.H., Oh, I.-J., Song, F., Lee, V.H.F., Su, W.-C., Chen, Y.-M., Chang, G.-C., Chen, K.-Y., Huang, M.-S., Yang, P.-C., Lin, H.-C., Xiang, Y.-B., Seow, A., Park, J.Y., Kweon, S.-S., Chen, C.-J., Li, H., Gao, Y.-T., Wu, C., Qian, B., Lu, D., Liu, J., Jeon, H.-S., Hsiao, C.-F., Sung, J.S., Tsai, Y.-H., Jung, Y.J., Guo, H., Hu, Z., Wang, W.-C., Chung, C.C., Lawrence, C., Burdett, L., Yeager, M., Jacobs, K.B., Hutchinson, A., Berndt, S.I., He, X., Wu, W., Wang, J., Li, Y., Choi, J.E., Park, K.H., Sung, S.W., Liu, L., Kang, C.H., Hu, L., Chen, C.-H., Yang, T.-Y., Xu, J., Guan, P., Tan, W., Wang, C.-L., Sihoe, A.D.L., Chen, Y., Choi, Y.Y., Hung, J.-Y., Kim, J.S., Yoon, H.-I., Cai, Q., Lin, C.-C., Park, I.K., Xu, P., Dong, J., Kim, C., He, Q., Perng, R.-P., Chen, C.-Y., Vermeulen, R., Wu, J., Lim, W.-Y., Chen, K.-C., Chan, J.K.C., Chu, M., Li, Y.-J., Li, J., Chen, H., Yu, C.-J., Jin, L., Lo, Y.-L., Chen, Y.-H., Fraumeni, J.F., Liu, J., Yamaji, T., Yang, Y., Hicks, B., Wyatt, K., Li, S.A., Dai, J., Ma, H., Jin, G., Song, B., Wang, Z., Cheng, S., Li, X., Ren, Y., Cui, P., Iwasaki, M., Shimazu, T., Tsugane, S., Zhu, J., Jiang, G., Fei, K., Wu, G., Chien, L.-H., Chen, H.-L., Su, Y.-C., Tsai, F.-Y., Chen, Y.-S., Yu, J., Stevens, V.L., Laird-Offringa, I.A., Marconett, C.N., Lin, D., Chen, K., Wu, Y.-L., Landi, M.T., Shen, H., Rothman, N., Kohno, T., Chanock, S.J., Lan, Q., 2017. Association between GWAS-identified lung adenocarcinoma susceptibility loci and EGFR mutations in never-smoking Asian women, and comparison with findings from Western populations. Hum. Mol. Genet. 26, 454–465. https://doi.org/10.1093/hmg/ddw414.
Simon, N.M., Smoller, J.W., McNamara, K.L., Maser, R.S., Zalta, A.K., Pollack, M.H., Nierenberg, A.A., Fava, M., Wong, K.-K., 2006. Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. Biol. Psychiatry 60, 432–435. https://doi.org/10.1016/j.biopsych.2006.02.004.
Valdes, A.M., Deary, I.J., Gardner, J., Kimura, M., Lu, X., Spector, T.D., Aviv, A., Cherkas,

L.F., 2010. Leukocyte telomere length is associated with cognitive performance in healthy women. Neurobiol. Aging 31, 986–992. https://doi.org/10.1016/j.neurobiolaging.2008.07.012.

Wei, Y. Bin, Martinsson, L., Liu, J.J., Forsell, Y., Schalling, M., Backlund, L., Lavebratt, C., 2016. hTERT genetic variation in depression. J. Affect. Disord. 189, 62–69. https://doi.org/10.1016/j.jad.2015.09.025.

Weinrich, S.L., Pruzan, R., Ma, L., Ouellette, M., Tesmer, V.M., Holt, S.E., Bodnar, A.G., Lichtsteiner, S., Kim, N.W., Trager, J.B., Taylor, R.D., Carlos, R., Andrews, W.H., Wright, W.E., Shay, J.W., Harley, C.B., Morin, G.B., 1997. Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT. Nat. Genet. 17, 498–502. https://doi.org/10.1038/ng1297-498.

Weng, Q., Du, J., Yu, F., Huang, T., Chen, M., Lv, H., Ma, H., Hu, Z., Jin, G., Hu, Y., Shen, H., 2016. The known genetic loci for telomere length may be involved in the modification of telomeres length after birth. Sci. Rep. 6, 38729. https://doi.org/10.1038/srep38729.

World Health Organisation, 2016. ICD-10 Version:2016. Who. https://doi.org/10.1177/1071100715600286.

Xu, X., Chen, X., Zhang, X., Liu, Y., Wang, Z., Wang, P., Du, Y., Qin, Y., Chen, Z.-J., 2017. Impaired telomere length and telomerase activity in peripheral blood leukocytes and granulosa cells in patients with biochemical primary ovarian insufficiency. Hum. Reprod. 32, 201–207. https://doi.org/10.1093/humrep/dew283.

Yaffe, K., Lindquist, K., Kluse, M., Cawthon, R., Harris, T., Hsueh, W.-C., Simonsick, E.M., Kuller, L., Li, R., Ayonayon, H.N., Rubin, S.M., Cummings, S.R., Health ABC Study, 2011. Telomere length and cognitive function in community-dwelling elders: findings from the Health ABC study. Neurobiol. Aging 32, 2055–2060. https://doi.org/10.1016/j.neurobiolaging.2009.12.006.