Chapter 9

Depression, telomeres and mitochondrial DNA: between-person and within-person associations from a 10-year longitudinal study

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ABSTRACT

Background Alterations in cellular aging, which can be indexed by leukocyte telomere length (LTL) and leukocyte mitochondrial DNA copy number (mtDNAcn), might partly account for the increased health risks in persons with depression. While some studies indeed found cross-sectional associations of depression with LTL and mtDNAcn, longitudinal associations remain unclear. This 10-year longitudinal study examined between-person and within-person associations of depressive symptoms with LTL and mtDNAcn in a large community sample.

Methods Data are from years 15, 20, and 25 follow-up evaluations in 977 subjects from the Coronary Artery Risk Development in Young Adults (CARDIA) study. Depressive symptoms (years 15, 20, 25) were assessed with the Center for Epidemiologic Studies Depression (CES-D) scale; LTL (years 15, 20, 25) and mtDNAcn (years 15, 25) were measured in whole blood by qPCR. With mixed models analyses, we explored between-person and within-person associations between CES-D scores and cellular aging markers.

Results Having high levels of depression throughout the 10-year timespan, averaged over the three time points, was associated with shorter average LTL over 10 years ($B=-3.6$; $p=.038$), in sociodemographic-adjusted analyses. However, no within-person associations were found between depression and LTL at each year ($B=-0.6$; $p=.635$). Additionally, we found no between-person ($B=-0.0$; $p=.994$) or within-person ($B=0.5$; $p=.423$) associations between depressive symptomatology and mtDNAcn.

Conclusions Our findings suggest that the relationship between depressive symptoms and LTL is based on between-person differences rather than a dynamic and direct within-person relationship. In this study we found no evidence for an association between depressive symptoms and mtDNAcn.

Key words: telomere length, mtDNA copy number, cellular aging, depression, depressive symptoms

INTRODUCTION

Persons with depression have increased somatic health risks (1,2) which may partly be explained by processes of accelerated cellular aging, including deterioration of telomere maintaining mechanisms and alterations in mitochondrial functioning (3). Physiological stress systems that are known to be dysregulated in the depressed population (4-6) may eventually lead to such cellular damage, ultimately contributing to increased risks for developing age-related somatic conditions. Two increasingly studied markers of cellular aging are telomere length and mitochondrial DNA copy number. Telomeres are DNA-protein complexes that cap chromosomal ends and promote chromosomal stability (7). Telomere length naturally decreases progressively over time – unless counteracted upon by the ribonucleoprotein cellular enzyme telomerase that adds telomeric DNA (8) – and is therefore strongly associated with age (9,10). When telomeres reach a critical short length, their function declines and cells progress into senescence or apoptosis. Short telomere length indeed correlates with the incidence of various aging-related somatic diseases such as cardiovascular disease or type II diabetes (11,12).

Mitochondrial function is also altered during aging; possibly as a result of an accumulation of damage to the mitochondrial genome. Mitochondria are cellular energy-generating organelles that play an important role in adenosine triphosphate (ATP) production and regulation of apoptosis (13). The mitochondrial genome – or mitochondrial DNA (mtDNA) – is a circular DNA molecule containing genes that encode essential components for ATP synthesis via oxidative phosphorylation (14). Every cell has hundreds to thousands of mitochondria in their cytoplasm, each containing two to ten copies of mtDNA (15). A sufficient number of mtDNA copies is found to be essential for healthy cellular function (16). The production of reactive oxygen species (ROS), a necessary by-product of oxidative phosphorylation, creates increasing oxidative damage and alterations of mtDNA (17) postulated to promote development of aging-related diseases (18,19) and pathophysiology of the central nervous system (20,21). Decreased number of leukocyte mtDNA per cell (i.e., mtDNA copy number) has been cross-sectionally associated with several somatic conditions, such as Parkinson's disease (22), breast cancer (23), hyperlipidemia (24) and metabolic syndrome (25). However, associations between somatic diseases and (possibly compensatory) excess mtDNA proliferation also have been reported (26). Recent research indicates co-regulation of telomere and mitochondrial processes (27,28), suggesting that both telomeres and mitochondria are functionally linked and associated with the aging process (29).

An increasing number of studies have examined leukocyte telomere length (LTL) and, to a lesser extent, mtDNA copy number (mtDNAcn) in relation to depression. Recent meta-analyses found a significant association between short LTL and depression (30,31), establishing a cross-sectional relation between LTL and clinically diagnosed depression.

Evidence of short LTL in studies using continuous self-reported measures of depressive symptomatology (and not necessarily a clinical diagnosis) remains mixed. Several large-scale studies found no effect (32,33), while some smaller studies are suggestive of a dose-response relationship between LTL and depressive symptoms in the general population (34,35). Few studies have examined whether mtDNAcn is associated with depression and findings are mixed (36). While two studies found a negative association with mtDNAcn and depressive symptomatology (37,38), another study failed to find any association (39). In contrast, two studies found positive associations between mtDNAcn and lifetime depression (40,41). Overall, it is thus unclear to what extent LTL and mtDNAcn are related to depressive symptomatology.

The lack of consistency of the relationships between cellular aging markers and depression perhaps rests in the varied possible ways to conceptualize and operationalize depression. Some studies observed continuous depressive symptoms, others looked at cut-offs and yet other studies included current or lifetime diagnoses of depression. Further, not all studies adjusted for important covariates such as lifestyle and somatic health, and the vast majority investigated merely the cross-sectional relation between cellular aging and depression. Thus, most studies up until now only had the opportunity to look at snapshots of the complex relationship between depression and markers of cellular aging, leaving the field with a scattered and incomplete picture. Accordingly, examinations of numerous biological and clinical variables on multiple time points are needed to increase the understanding of the temporal trajectory of cellular aging and depression.

The current large-scale study (N=977) analyzed data on LTL, mtDNAcn and depressive symptomatology, self-reported diagnosis, and antidepressant medication in a community-based sample of the Coronary Artery Risk Development in Young Adults (CARDIA) study. Data was available from three time points over a 10-year period. With this longitudinal approach, we had the opportunity to test both the long-term associations between depressive characteristics and cellular aging markers over the 10-year period and the within-person associations at each time point. Also, by looking at group-by-time interactions, we were able to test whether the trajectories of LTL or mtDNAcn over time were a function of depressive symptomatology.

METHODS AND MATERIALS

Study sample

Data are from participants of the Coronary Artery Risk Development in Young Adults (CARDIA) Study. The CARDIA study examined the development and determinants of clinical and subclinical cardiovascular disease and their risk factors, and started in 1985 with a group of 5115 black and white men and women aged 18-30 years. The participants

were selected so that there would be approximately the same number of people in subgroups of race, sex, education (high school or less and more than high school) and age (18-24 and 25-30) in each of 4 centers: Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. All participants were asked to participate in follow-up examinations during 1987-1988 (Year 2; subsequently Y2), 1990-1991 (Y5), 1992-1993 (Y7), 1995-1996 (Y10), 2000-2001 (Y15), 2005-2006 (Y20), and 2010-2011 (Y25). A majority completed follow-up examinations (90%, 86%, 81%, 79%, 74%, 72%, and 72%, respectively). Details of the study's design and procedures have been published elsewhere (42). The current study reports data from an ancillary study, for which participants were selected if they had stored DNA from whole blood available at Y15, Y20 and Y25 and also coronary artery calcification data on the same examinations. For those 1000 participants, two markers of cellular aging were determined: LTL was examined at Y15, Y20 and Y25; and mtDNAcn was measured at Y15 and Y25. Overall, data on LTL, mtDNA and depressive symptomatology was complete for 977 subjects at the three time points.

Depression

The Center for Epidemiologic Studies Depression (CES-D) scale was administered to measure depressive symptoms. The CES-D is a validated and reliable 20-item self-report scale designed to detect the presence of depressive symptoms during the past week (43). The standard cut-off point of 16 or more was used to indicate the presence of clinically relevant depressive symptoms. For the purpose of the current study, we included CES-D scores administered at Y15, Y20 and Y25 in this study. Further, in Y15, Y20 and Y25 participants were asked whether they were ever clinically diagnosed with depression (i.e. Has a doctor or nurse ever said that you have depression?); and if yes, if they had had this diagnosis in the past year. Participants were also asked whether they were currently taking medications for depression.

Blood draw and sample preparation

Blood samples were drawn by venipuncture, according to a standard protocol (42), in the morning after an overnight fast (>8 hours) using EDTA-containing tubes. Persons were asked not to smoke or perform heavy physical activity for two hours before their examination visit. Blood was taken from participants, and centrifuged within 2 hours of blood draw. Whole blood was stored at −70°C until shipped on dry ice to the Genetics laboratory of Dr. Fornage at the University of Texas Health Sciences Center (Houston, TX, USA). DNA for TL was prepared using Gentra Puregene Cell kit (QIAGEN, Valencia, CA, USA).

Leukocyte telomere length

DNA samples were shipped by the CARDIA study in 96-well plates to UCSF Blackburn lab. All DNA samples were stored in their original plates in a -80°C freezer upon arrival. Of the original DNA samples, 5ul was diluted with 10 ul of sterile H_2O to reach final concentration of 33.3 ng/ul. The diluted DNA samples were plated in 96-well plates and stored in a -80°C freezer. DNA samples were thawed on ice on the day of the assay and transferred to 384-well plates. The telomere length measurement assay was adapted from the published original method (44). Telomere length values were measured from DNA by a quantitative PCR (qPCR) assay that determines the ratio of telomere repeat copy number to single-copy gene copy number (T/S) ratio in experimental samples as compared with a reference DNA sample. Higher T/S ratio signifies longer mean telomere length.

Primers for the telomere PCR (T runs) are *tel1b* [5'-CGGTTT(GTTTGG)$_5$GTT-3'], used at a final concentration of 100 nM, and *tel2b* [5'-GGCTTG(CCTTAC)$_5$CCT-3'], used at a final concentration of 900 nM. The primers for the single-copy gene (human beta-globin) PCR (S runs) are *hbg1* [5' GCTTCTGACACAACTGTGTTCACTAGC-3'], used at a final concentration of 300 nM, and *hbg2* [5'-CACCAACTTCATCCACGTTCACC-3'], used at a final concentration of 700 nM. A total of 977 participants had complete LTL data at three time points.

Mitochondrial DNA copy number

Mitochondrial DNA copy number was measured by qPCR. A 69 bp fragment within the ND1 gene in mtDNA (nucleotides 3485-3553) and a 87 bp fragment within the RNase P gene in the nuclear DNA were simultaneously amplified by a multiplex TaqMan-based qPCR reaction adapted from Krisnan et al. (45). This was used to determine the relative number of mtDNA copies per diploid nuclear genome (i.e., per cell). The VIC-labelled probe and primer set for RNase P were obtained from Life Technologies (cat#4403328). The primer and probe sequences for ND1 (IDT) are: ND1-forward 5'-CCCTAAAACCCGCCACATCT-3', ND1-reverse 5'-GAGCGATGGTGAGAGCTAAGGT-3', ND1-FAM probe 5' FAM-CCATCACCCTCTACATCACCGCCC-TAMRA-3'.

The reaction contained 12.5 ng of genomic DNA, 100nM of ND1 probe, 300 nM of ND1-formard primer and ND1-reverse primer each, 1X RNase P copy number Reference Assay, 1X LightCycler® 480 Probe Master (Roche, cat# 04902343001) in a 10 ul reaction. All samples were run in triplicate wells in 384-well plates in a Roche Lightcyler 480. PCR conditions are 95°C 10 min 45 cyles of 95 °C 10 sec, 60°C 30 sec, 72°C 1 sec with data acquisition at 72°C. Crossing point (CP) for each well is derived by the LightCycler 480 program using the second derivation method. Relative copy number is calculated by the following formula: relative mtDNA copy number/diploid genome=POWER[2, (CP_{ND1}-

CP_{RNaseP})]*2. Inter-assay CV using 85 CARDIA samples was 3.4% and intra-assay CV using 4 control DNA samples, averaged from all 21 CARDIA sample plates, was 1.7%.

Covariates

Age, sex [0=female; 1=male] and race [0=black; 1=white] were determined with a telephone interview before inclusion. All other covariates were recorded at each time point. Education [0=completed high school or less; 1=completed high school & college; 2=at least some post-college education] was determined by self-report questionnaire. Participants were asked whether they currently smoked or had smoked in the past [0=never smoked; 1=quit smoking; 2=current smoker]. Alcohol consumption was categorized based on the average number of drinks per week [0=non-drinkers; 1=moderate drinkers (women: 1-10 drinks/week; men: 1-15 drinks/week); 2=heavy drinkers (women: >10 drinks/week; men >15 drinks/week)]. Based on an interviewer-administered physical activity history, total activity was computed as the sum of frequency and intensity scores for 13 categories of vigorous and moderate activity over the previous 12 months (46). Participants reported whether they had ever been diagnosed with any of the following major or chronic health conditions: heart problems, diabetes, liver disease, migraine headaches, peripheral vascular disease, cancer, thyroid problem, kidney failure/dialysis/transplant, digestive disease, ulcer, gout, asthma or chronic bronchitis, epilepsy, pneumonia, tuberculosis, emphysema, stroke or TIA, multiple sclerosis or HIV. The number of conditions was summed. BMI was calculated as measured weight in kilograms divided by height in meters squared and then divided into categories [0=normal (18.5–24.9); 1=underweight (<18.5); 2=overweight (25.0–30.0); 3=obese (>30.0)].

Statistical analyses

Data were analyzed using SPSS version 20.0. Due to the repeated nature of measurement of our predictors and outcomes, non-independence between repeated measures was present. Mixed models analyses were completed with restricted maximum likelihood estimation, random intercepts and fixed slopes, thereby accounting for non-independence in the data. First, mixed model analyses, using all data available, tested associations between LTL and mtDNAcn and CES-D cutoff score [0=CES-D<16; 1=CES-D≥16], current use of antidepressant medication [0=no; 1=yes] and self-reported depression [0=no; 1=yes]. Next, grand-centering and person-centering the CES-D scores allowed the examination of the between-person (averages of the years) and within-person (at each time point) relationships between depressive symptomatology and cell aging markers. Grand-centered means are computed first by calculating each person's mean CES-D scores over the three time points and then subtracting each person's mean from the total sample's mean. This would indicate that the average over the 10-year

period was associated with the average LTL or mtDNAcn over the 10 years. Person-centered means are calculated by subtracting each person's CES-D score at each time point from their own average calculated with the three time points (or two for mtDNA), thus allowing the examination of whether higher or lower CES-D scores at each time point is associated with LTL or mtDNAcn. This would suggest a within-person association between depression and LTL or mtDNAcn.

Depression characteristics (CES-D cutoff score, antidepressant use, self-reported diagnosis, grand-centered CES-D, person-centered CES-D) were entered separately as predictor variables in mixed models analyses with LTL of mtDNAcn as outcome variables. Based on a priori defined covariates we created two models for adjustment: model 1 was adjusted for age, sex, race and education (sociodemographics); and model 2 additionally adjusted for lifestyle and somatic diseases. Dummy variables for time were entered into the models [1, 2, 3] corresponding with the years 15, 20 and 25. Subsequently, interaction terms of person/grand-centered mean-by-time were included: an interaction effect of person-centered means with time tested whether the association between depression and LTL or mtDNAcn differed between the time points; and by interacting grand-centered CES-D with time we tested whether depression over 10 years was related to LTL or mtDNAcn change over time. Effect modification by sex and race was tested by including depression characteristic-by-sex and depression characteristic-by-race interaction terms into the models.

RESULTS

Sample characteristics over the three time points are displayed in Table 1. Participants had an average age of 40.5 years (range 32-51) at Y15 and were examined consecutively 5 and 10 years later. About 65% of the total sample was female and nearly 60% was white. The self-reported CES-D depressive symptomatology scores were on average below the clinical cutoff. However, a considerable proportion of the sample (14-16%) reported a score above 16 at any of the three time points, indicative of clinically relevant depressed mood. This prevalence rate resembled earlier research on lifetime depression (47). The CES-D scores at Y15, Y20 and Y25 were inter-correlated ($r>.52$; all p-values $<.001$). The three LTL measures were also significantly correlated ($r>.45$; all p-values $<.001$), as was mtDNAcn at Y15 and Y25 ($r=.46$, $p<.001$). LTL and mtDNAcn correlated positively but modestly at Y15 ($r=.147$; $p<.001$) and Y25 ($r=.084$; $p=.008$). Further, both LTL ($B=-51.6$; $p<.001$) and mtDNAcn ($B=-5.0$; $p<.001$) were negatively related to age (see Figure 1 for temporal trajectories). Since recruitment of CARDIA participants was originally balanced for sex and race, age-adjusted means of CES-D, LTL and mtDNAcn by sex and race groups are presented in Table 2. Analyses of covariance showed that men and white individuals

overall had shorter LTL and lower mtDNAcn, and depressive symptoms were higher for women (Table 2). No sex-by-race interactions were found.

Table 1. Sample characteristics at three time points (N=977)

	Y15	Y20	Y25
Sociodemographics			
Age (mean ± sd)	40.5 (3.6)	45.4 (3.6)	50.4 (3.6)
Sex (% female)	65.4	-	-
Race (% white)	59.4	-	-
Education (%)			
Completed high school or less	19.0	21.3	20.0
Completed high school & college	60.2	56.3	55.0
Some postcollege education	20.8	22.4	25.0
Depression characteristics			
CES-D score (mean ± sd)	8.5 (7.3)	9.0 (7.8)	9.0 (7.4)
CES-D clinical cutoff (% ≥16)	13.7	15.4	16.0
Depression past year (% yes)	8.8	11.4	13.2
Depression lifetime (% yes)	15.6	18.7	21.3
Current antidepressant medication (% yes)	6.9	10.2	11.1
Lifestyle and health factors			
Body mass index (%)			
Underweight	1.3	0.6	0.5
Normal	30.2	27.9	23.9
Overweight	35.8	33.0	31.6
Obese	32.7	38.5	44.0
Smoking status (%)			
Never smoked	62.4	63.0	63.2
Former smoker	18.7	21.5	23.1
Current smoker	19.0	15.5	13.8
Alcohol status (%)			
Non-drinker	27.6	24.8	22.7
Moderate drinker	63.1	64.9	66.4
Heavy drinker	9.3	10.3	10.9
Number of somatic diseases (mean ± sd)	0.9 (1.1)	1.0 (1.2)	1.4 (1.5)
Physical activity (mean ± sd)	336 (274)	334 (273)	335 (272)
Cellular aging markers			
Base pairs (mean ± sd)	5596 (480)	5681 (451)	4971 (276)
mtDNA copy number (mean ± sd)	513 (141)	NA	459 (125)

Abbreviations: CES-D = Center for Epidemiologic Studies, Depression Scale; mtDNA = mitochondrial DNA; NA = not available; sd = standard deviation

Table 2. Descriptives of depressive symptoms (CES-D) and cellular aging markers (LTL & mtDNAcn) by sex & race groups

		Black male (N=102)	Black female (N=295)	White male (N=236)	White female (N=344)	Sex	Race	Sex * Race
Variable	Year	mean (SE)	mean (SE)	mean (SE)	mean (SE)	p-value	p-value	p-value
CES-D	15	8.1 (0.7)	10.3 (0.4)	6.9 (0.5)	8.0 (0.4)	<.001	<.001	.271
	20	8.9 (0.8)	11.0 (0.5)	7.6 (0.5)	8.3 (0.4)	.003	<.001	.199
	25	8.3 (0.7)	10.6 (0.4)	8.2 (0.5)	8.6 (0.4)	.010	.001	.053
LTL	15	5598.3 (47.5)	5653.0 (27.9)	5539.5 (31.1)	5585.1 (25.8)	.065	.021	.893
	20	5700.4 (44.4)	5764.4 (26.1)	5604.7 (29.1)	5656.9 (24.2)	.015	<.001	.853
	25	4961.1 (27.3)	5007.1 (16.0)	4935.1 (17.9)	4968.0 (14.9)	.019	.024	.739
mtDNAcn	15	523.4 (13.5)	566.1 (7.9)	468.5 (8.8)	493.9 (7.4)	<.001	<.001	.373
	25	474.8 (11.8)	504.9 (6.9)	399.6 (7.7)	454.6 (6.4)	<.001	<.001	.126

Note. mean and SE are age-adjusted; p-values from separate ANCOVAs represent main effects of sex and race and a sex*race interaction effect
Abbreviations. CES-D = Center for Epidemiologic Studies, Depression Scale; LTL = leukocyte telomere length; mtDNAcn = mitochondrial DNA copy number; SE = standard error

Associations of depression characteristics with LTL and mtDNAcn

Descriptive associations

Estimated LTL and mtDNAcn means of both CES-D cutoff groups over time are displayed in Figure 1A and 1B, respectively. Those with a CES-D score above the cutoff of 16, indicative of clinically relevant depressed mood, had shorter average LTL throughout three time points in mixed model analyses (B=-49.8; p=.015, Table 3, Figure 1A). This association remained significant after further adjustment for somatic health and lifestyle variables. No associations between CES-D cutoff and mtDNAcn were found over the two time points (p=.610, Table 3, Figure 1B). Further, current use of antidepressant medication or self-reported depression diagnosis were not associated to LTL or mtDNAcn (Table 3).

Mixed models for between-person associations between CES-D and LTL or mtDNAcn over 10 years

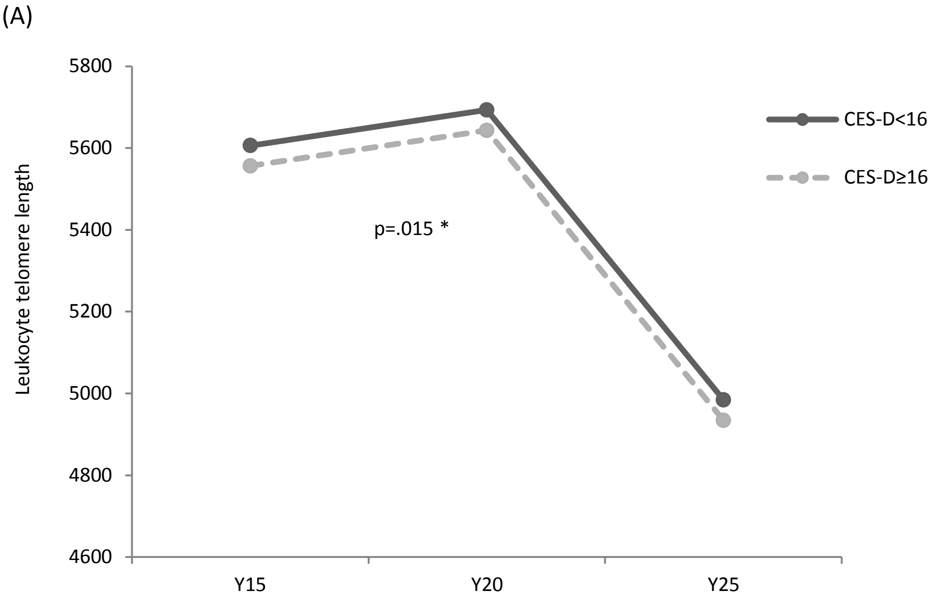
Mixed model analyses showed that grand-centered CES-D means were negatively related to LTL over the 10 years (B=-4.2; p=.016; Table 3). Thus, a person who had a high CES-D score averaged over the three time points tended to have shorter LTL. The association remained significant after adjustment for somatic health (p=.036) but became borderline significant when adjusted for lifestyle (p=.061). We found no overall interaction of grand-centered mean-by-time (p=.383), showing that a person's average CES-D score over 10 years was unrelated to a change in LTL. Further, sex (p=.993) and race (p=.775) were not identified as moderators. We found no associations of mtDNAcn with grand-centered CES-D over two time points (Table 3), and no interaction of grand-centered CES-D means-by-time.

Mixed models for within-person associations between CES-D and LTL or mtDNAcn at each time point

Mixed model analyses showed that person-centered CES-D scores were not associated to LTL, adjusted for sociodemographics and time (B=-0.2; p=.737; Table 3), suggesting that CES-D scores were not concurrently related to LTL. In other words, when a person's self-reported depressive symptoms increased at any time point from their own average, LTL did not change in parallel. No interactions of person-centered means-by-time were found (time 1 = reference; time 2=-2.3; p=.567; time 3=6.5; p=.107), thus associations between depression and LTL did not differ between the time points, although there was a trend towards a weaker effect at the third time point (Y25). No associations were found between person-centered CES-D and mtDNAcn (Table 3), indicating the absence of a within-person association between mtDNAcn and depressive symptomatology.

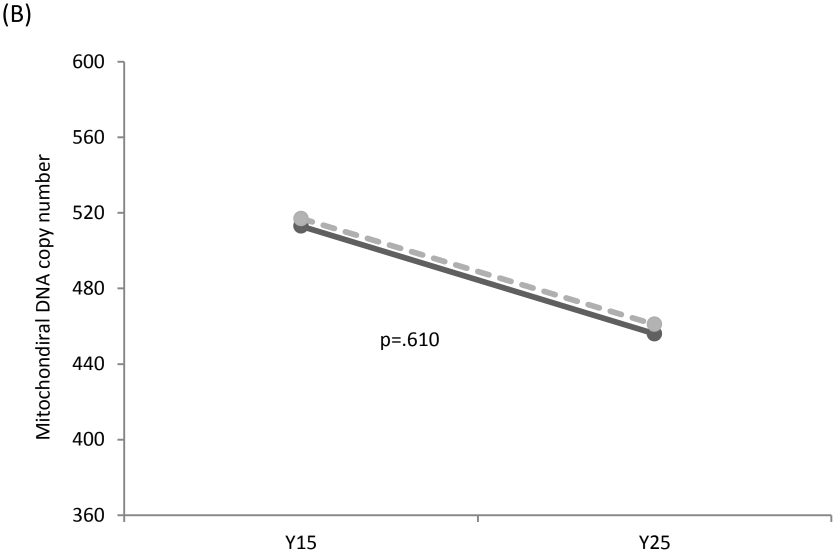
Figure 1. Trajectory of (A) leukocyte telomere length and (B) mtDNA copy number over time by CES-D cutoff groups

(A)



* group difference across 3 time points

(B)



Note. Estimated means from mixed model analyses, adjusted for age, sex, race, education and time

Table 3. Associations of depression characteristics with cellular aging markers across multiple time points

	Leukocyte telomere length [Y15+Y20+Y25]		Mitochondrial DNA copy number [Y15+Y25]	
	B [95% C.I.]	p-value	B [95% C.I.]	p-value
CES-D cutoff				
model 1	-49.8 [-89.9 – -9.6]	.015	4.2 [-11.9 – 20.3]	.610
model 2	-54.3 [-97.2 – -11.4]	.013	3.9 [-13.2 – 20.9]	.655
model 3	-52.9 [-96.0 – -9.9]	.016	6.7 [-10.4 – 23.9]	.442
Antidepressant use				
model 1	6.5.1 [-44.0 – 57.0]	.800	7.4 [-12.7 – 27.4]	.472
model 2	13.4 [-40.0 – 66.8]	.623	8.1 [-12.9 – 29.2]	.448
model 3	19.1 [-34.5 – 72.7]	.485	9.5 [-11.6 – 30.6]	.377
Self-reported diagnosis				
model 1	-12.0 [-57.7 – 33.7]	.608	9.0 [-9.1 – 27.1]	.329
model 2	-5.8 [-54.7 – 43.1]	.817	11.2 [-8.2 – 30.7]	.257
model 3	-2.6 [-51.6 – 46.4]	.918	12.2 [-7.3 – 31.7]	.219
Grand-centered CES-D				
model 1	-4.2 [-7.5 – -0.8]	.016	-0.2 [-1.2 – 0.9]	.737
model 2	-3.8 [-7.3 – -0.3]	.036	-0.0 [-1.1 – 1.1]	.965
model 3	-3.4 [-7.0 – 0.2]	.061	0.3 [-0.8 – 1.4]	.626
Person-centered CES-D				
model 1	-0.8 [-3.3 – 1.8]	.551	0.4 [-0.8 – 1.7]	.497
model 2	-1.1 [-3.7 – 1.6]	.432	0.6 [-0.8 – 1.9]	.407
model 3	-1.1 [-3.8 –1.6]	.417	0.7 [-0.6 – 2.0]	.316

Model 1: adjusted for age at Y15, sex, race, education & time
Model 2: additionally adjusted for somatic diseases
Model 3: additionally adjusted for smoking, alcohol, BMI & physical activity
Abbreviations: CES-D = Center for Epidemiologic Studies, Depression Scale; C.I. = Confidence Interval

DISCUSSION

This large cohort study captured LTL, mtDNAcn and depressive symptomatology over 10 years, thereby providing novel insights in the longitudinal trajectories of associations between markers of cellular aging and depression. First, we showed that persons with higher depressive symptoms over 10 years had on average shorter LTL over the same timespan than those with lower depression levels. However, when persons increased their depressive symptoms level at one time point compared to their individual average, this was not accompanied by a concurrent decrease in LTL. This suggests that the association of depression with short LTL is best explained as a between-person effect, rather than a dynamic within-person relationship. Second, the absence of an interaction with time showed that LTL shortening was not accelerated in those with high levels of depression. Third, we found no evidence for an association between depressive symptoms and mtDNA copy number (mtDNAcn), whether we examined associations averaged for the 10 years, at each time point, or over time.

To date, an increasing number of studies provided evidence for the cross-sectional association of depression and LTL (30); however, the nature of this relationship remained unclear. Here, we showed that persons with higher levels of depressive symptomatology have shorter LTL, but importantly, increases in depressive symptoms within an individual did not necessarily translate to a shorter telomere length at the same time point. Moreover, LTL attrition over time was not accelerated in those with high levels of depression. These findings add to a growing literature on the relationship between depression status and LTL changes over time, supporting two studies that have not evidenced a dynamic relationship (48,49) – but not another study that did find greater LTL decrease in depression and anxiety disorder patients (50). How, then, do we reconcile the overall significant between-person relationship of depression and LTL with the lack of a relationship within-persons at each time point? A first possibility is that a person's first episode with clinically relevant symptoms causes accelerated LTL shortening, and this shortened LTL is, despite eventual remission of the depression, never able to fully recover to the average of never-depressed individuals. In other words, one depressive episode might leave a long-lasting cellular "scar". This may partly be explained by the finding that persons who have had a depressive episode often continue to have elevated depressive symptomatology after remission (51,52), and consequently shorter average LTL. This explanation is supported by findings in the Netherlands Study of Depression and Anxiety, where remitted MDD patients showed considerable residual depressive symptomatology along with short LTL (53). An alternative explanation is that a 'third factor' might have a role in both depression development and LTL shortening. One proposed factor is inflammation: a large body of research suggests that prolonged activation of the immune system enhances vulnerability to depression. Elevated levels of pro-inflammatory

cytokines and acute phase proteins, such as interleukin-6 and C-reactive protein (54), might directly evoke depressogenic states (55). Further, high cytokine levels in the brain might impact microglia activation (56), neurotransmitter metabolism and reduce neurogenesis (particularly in the hippocampus), thereby contributing to the pathophysiology of depression (57). Interestingly, an *in vitro* study provided evidence that pro-inflammatory markers may also directly accelerate telomere shortening (58), which is further supported by similar associations *in vivo* (59,60). Another underlying third factor might include genetics: overlapping genetic or epigenetic effects might influence both depression and LTL, for example a genetic variation in hTERT (61). Last, certain lifestyle factors might also both increase the vulnerability for depression and directly impact LTL (e.g., diet or smoking (62)).

Results of the current study suggest an absence of a relationship between depressive symptomatology and mtDNAcn. None of the depression characteristics in this study were associated with mtDNAcn. Existing literature has reported conflicting findings. A recent large scale (N>10,000) study by Cai et al. (40) found a positive association between recurrent major depression and the amount of mtDNA in saliva. Likewise, Tyrka et al. (41) found higher whole blood mtDNAcn for 59 persons with lifetime depression compared to 113 controls. He et al. (39), on the other hand, found no difference in leukocyte mtDNAcn between 210 depressed patients and 217 controls, whereas two other studies in found a negative association with the number of leukocyte mtDNA copies and depressive symptomatology in ~130 community-dwelling elderly women (37,38). Various ways of operationalizing depression might account for the conflicting outcomes: two studies used the Geriatric Depression Scale-15 (37,38), others current or lifetime DSM-IV diagnoses (39-41), while the current study used the CES-D scale. It is notable that the two studies that found a positive relationship both used DSM-IV based lifetime depression diagnoses, which may suggest that mtDNAcn differences are mainly present in those with clinically diagnosed depression rather than elevated (but subclinical) depressive symptomatology. Another explanation for the inconsistent findings could be the differences in biological material used, including whole blood, purified total leukocytes, mononuclear cells (PBMCs) and saliva, since these cell types have different mtDNAcn dynamics (63).

The conflicting results of mtDNAcn and depression research, further, show the relative novelty of mtDNAcn as a marker of cellular aging and health in general. Recent studies showed associations between low mtDNAcn and various age-related health outcomes such as cognitive performance, physical strength (64), diabetes (65) and mortality (58); accordingly, lower mtDNAcn seems to be related to worse health. However, others suggest that the number of copies of mtDNA per cell may increase to compensate for DNA damage (59). In this theory, mtDNAcn might increase

disproportionately to compensate for mitochondrial electron transport chain dysfunction (60); and higher mtDNAcn may represent a marker of poor mitochondrial health, or mitochondrial allostatic load (6). This might explain the previously discussed findings of higher mtDNAcn in relation to depression (40,41). However, bearing in mind the negative association with age (37,66-68) and the positive relation between LTL and mtDNAcn reported by the current study as well as several earlier studies (27,69-71), lower mtDNAcn appears to be associated with worse physical and psychological health.

This study is the first study to report on LTL, mtDNAcn and depressive symptomatology over 10 years including multiple time points. A major strength is that cellular aging markers from different years were all assayed together in the same lab, randomized over batches, thus avoiding potential batch-related noise. Other strengths are the large sample size with well-characterized persons, the inclusion of a wide variety of possible covariates and ways of operationalizing depression. This study included continuous depressive symptomatology, a cutoff indicative of clinically relevant depressive symptoms, information on antidepressant use and self-reported physician diagnosed depression. Findings of the current study, however, must be interpreted in light of some limitations. First, self-reported depression diagnosis was not found to be associated with cellular aging, and only modestly correlated with CES-D cutoff (r=.30). This indicates that retrospective self-reported physician diagnosed depression might not be a suitable research measure for depression. Further, this study had a limited number of within-person assessments, such that variability within each participant might not have been large enough to show the minute changes in LTL that would accompany large changes in depressive symptomatology. Future studies should preferably capture LTL-depression associations in over more than three assessments. Something else to note is that, provided CARDIA's mean age at year 15 is 40 and that the average age of depression onset is 32 years, perhaps our assessments fell outside the optimal age range to capture the within-person relationship between depressive symptomatology and LTL. Another concern of the present study is the somewhat unexpected trajectory of LTL over 10 years: over the first 5 years the study sample showed rather stable LTL, while it decreased considerably in the last 5 years. However, the average yearly attrition rate of ~50 bp/year was not aberrant from other longitudinal studies (72) and consistent age, sex and race associations with LTL confirmed the validity at all three time points. Last, we assessed LTL and mtDNAcn in leukocytes from whole blood, as in most epidemiological studies. A limitation of using unsorted leukocytes is that it consists of different cell types with different phenotypes, telomere length, and mtDNA copy number. A recent study found different LTL attrition rates for T-cells, B-cells and monocytes, which makes it possible that LTL differences are due to a redistribution of cell types (73). Further mtDNAcn differs

substantially between whole blood and leukocytes populations (e.g., lymphocytes) (74), thus the lack of cell type sorting could be considered a limitation of the present study.

In conclusion, this study reports novel insights in the longitudinal trajectories of depressive symptomatology with cellular aging markers LTL and mtDNAcn. We provide evidence for a long-term, but non-dynamic, relationship between depression and LTL. Our results are suggestive of a between-person effect, rather than a within-person relationship. Future research is needed to elucidate whether this is the consequence of the failure of LTL repair mechanisms to recover after a depressive episode, thus leaving a long-lasting cellular “scar”; or whether an underlying third factor, for instance inflammation or shared genetic effects, impact both depression vulnerability and accelerate LTL shortening. This study found no evidence for a relation between depressive symptoms and mtDNAcn, possibly because mtDNAcn is mainly associated with clinically diagnosed depression rather than elevated subclinical depressive symptomatology, although this novel field remains to be further explored.

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Conflicts of interest

All authors declare that they have no conflicts of interest.

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