Supplementary Information

Measurement of mean relative telomere length

The method designed by Cawthon[1] determines mean telomere length across all chromosomes for all cells sampled. The method involves two quantitative PCR reactions for each subject; one for a single-copy gene (S) and the other in the telomeric repeat region (T). Given each of these reactions is compared to the within entire sample mean value before the (T)/(S) ratio is calculated, the ratio will equal 1 when the experimental sample is identical to the sample mean value. Thus, the ratio of one individual to another will correspond to the relative telomere lengths of their DNA. All DNA samples were run in triplicate for telomere and single-copy reactions at both age 26 and age 38 i.e., 12 reactions per Study member.

For both telomere and single-copy reactions, 15 µl PCRs were set-up using a QIAgility robotic pipettor (Qiagen, MD, USA) to ensure maximum pipetting accuracy. The telomere PCR contained the following reactants: 30 ng gDNA, 7.5 µl 2x SYBR Q-PCR ROX Mix (Qiagen), 0.27 pmol forward primer (Tel1; Sigma), 0.9 pmol reverse primer (Tel2; Sigma), 0.15 µl DMSO (Sigma), made up to 15 µl with dH_2O. Primer sequences were: tel 1, GGT TTT TGA GGG TGA GGG TGA GGG TGA GGG TGA GGG T; tel 2, TCC CGA CTA TCC CTA TCC CTA TCC CTA TCC CTA TCC CTA. The single-copy PCR contained the following reactants: 30 ng gDNA, 7.5 µl 2x SYBR Q-PCR ROX Mix (Qiagen), 0.3 pmol forward primer (36B4u; Sigma, MO, USA), 0.5 pmol reverse primer (36B4d; Sigma), 0.15 µl DMSO (Sigma), made up to 15 µl with dH_2O. Primer sequences were 36B4u: CAG CAA GTG GGA AGG TGT AAT CC;

36B4d, CCC ATT CTA TCA TCA ACG GGT ACA A. In both cases, thermalcycling was performed on an AB7900HT TaqMan real-time light cycler (Applied Biosystems, CA., USA) set up in real-time data acquisition mode. For the telomere reaction, cycling parameters were: initial incubation step at 95°C for 15 min followed by 30 cycles of 95°C for 15 s and 54°C for 2 min. For the single-copy reaction, cycling parameters were: initial incubation step at 95°C for 15 min, followed by 30 cycles of 95°C for 15 s and 58°C for 1 min. Primer concentrations were optimized for each batch in accordance with AB9700 Taqman guidelines (Applied Biosystems). Each plate contained a 1.68-fold serial DNA dilution of a reference sample used as a standard curve, ranging from 80 to 2.12 ng DNA per reaction. The reference sample consisted of a pool of DNA from 96 separate individuals. To reduce variance, DNA triplicates from the same individual, at both age-26 and age-38, were assayed on the same plate to avoid difference in conditions between plates confounding age comparisons. Sample grid position was preserved between telomere and single copy reactions to minimize experimental error due to positional effects (**Supplementary Figure S1**).

Quality control and data clean-up

Raw Ct data were initially assessed for assay success; Ct values for which all three replicates fell outside the range of the standard curve were re-assayed with their matching alternate-age sample by creating new dilutions and performing both telomere and single-copy reactions again. A proportion of samples were also re-assayed in this second round to give a measure of intra-plate (n = 26) and inter-plate (n = 139) variation. Samples which fell outside the range of the standard curve after both assay attempts were

removed from the final dataset (N=18). Then, triplicates were assessed for reliability; outliers within triplicate Ct values for each sample (telomere and single copy) were identified via Grubb's method (critical $Z = 1.15$) and excess in standard deviation (SD > 1); outliers were removed from subsequent relative ratio calculation. After removing outliers within triplicates, the average coefficient of variation (CV) for the triplicate Ct values was 0.81% for the telomere (T) and 0.48% for the single-copy gene (S).

Data acquisition and relative ratio calculation

Relative quantities of samples for each of the reactions were calculated using the procedure described by Pfaffl[2]. First, the slope of each standard curve (one/ plate/ reaction) was calculated using the formula $y = slope*x + intercept$, where y = Ct value and $x = \log_{(10)}$ input amount DNA (ng). Reaction efficiencies (E) were calculated from the standard curve slope using $E = 10^{(1/-slope)}$, where at 100% efficiency E = 2. Average E values for the telomere reaction were 2.178, and 2.072 for the single copy reaction. Next, relative quantities (RQs) for each sample were calculated using $RQ = E^{\Delta Ct}$ where ΔCt is the difference between the average within-plate Ct value and the mean triplicate Ct value of the individual sample. Sample RQ values were calculated for each reaction separately (T and S). Finally, the telomere length relative to the amount of single-copy transcript was calculated using the ratio RQ(T)/RQ(S) (NRQ) and finally normalized to the NRQ value of the 28.4 ng standard for each plate to give a final Calibrated, Normalized Relative Quantity (CNRQ). Intra-plate CNRQ values of replicate samples were significantly correlated ($r = .936$, $p < 0.001$).

Ruling out potential effects of white blood cell counts

Differences in leukocyte telomere length (LTL) between individuals may partly depend on differences in the composition of all leukocyte cell subpopulations in a given sample[3]. LTL is comprised of the average telomere length of all five immune cell subpopulations within a given sample (i.e., neutrophils, lymphocytes, monocytes, eosinophils and basophils). Telomere length may vary in different subpopulations of lymphocytes[3], and cell subpopulation composition may be influenced by a higher replicative rate of specific immune cells, as a result of more activated immune states (such as in internalizing disorder[4, 5]) at the time blood is drawn.

We measured white blood cell (WBC) counts at age 38 years (including counts of neutrophils, lymphocytes, monocytes, eosinophils and basophils) by flow-cytometry using a semiconductor laser to produce forward and lateral scattered light. All WBC counts were measured as $x10^9$ /L. CVs: WBC = 3%, neutrophils = 8%, lymphocytes = 8%, monocytes = 20%, eosinophils = 25% and basophils = 40%.

Supplementary Table S3 shows that, among men, higher lymphocyte count, higher agranulocyte count (both lymphocytes and monocytes), as well as total WBC count were significantly correlated with internalizing disorder from 11 to 38 years, while higher basophil count was correlated with shorter age-38 LTL. However, controlling for each of the WBC counts alone, and controlling for all WBCs simultaneously, did not alter the association between internalizing disorder from 11-38 years and LTL at age 38 years.

Supplementary Table S1: Description of age-38 clinical indicators of poor physical health.

Clinical Indicator	Description	Prevalence at age 38 Males	Females
Metabolic abnormalities	We assessed metabolic abnormalities by measuring 5 clinical indicators: obesity, high density lipoprotein (HDL) cholesterol level, triglyceride level, blood pressure, and glycated hemoglobin concentration.		
Obesity[1]	To determine obesity, we measured waist circumference (in centimeters). Study members were considered obese if their waist measurement was greater than 88 cm for women or greater than 102 cm for men.	15%,	25%
High-Density Lipoprotein level[1]	Study members were considered to have a low HDL cholesterol level if the value was 40mg/dL (1.04 mmol/L) or lower for men and 50 mg/dL (1.30 mmol/L) or less for women.	25%,	24%
Triglyceride level[1]	Study members were considered to have an elevated triglyceride level if their reading was 2.26 mmol/l or greater.	49%,	14%
Blood pressure[1]	Blood pressure (in millimeters of mercury) was assessed according to standard protocols[6]. Study members were considered to have high blood pressure if their systolic reading was 130 mm Hg or higher or if their diastolic reading was 85 mm Hg or higher.	37%,	16%
Glycated hemoglobin[2]	Glycated hemoglobin concentrations (expressed as a percentage of total hemoglobin) were measured by ion exchange high performance liquid chromatography (Variant II; Bio-Rad, Hercules, Calif) (coefficient of variation, 2.4%), a method certified by the US National Glycohemoglobin Standardization Program (NGSP) http://www.missouri.edu/~diabetes/ngsp.html). Study members were designated as having this health risk if their scores were greater than 5.7%, the cutoff for pre-diabetes.	22%,	13%
Cardiorespiratory fitness	Maximum oxygen consumption adjusted for body weight (in milliliters per minute per kilogram) was assessed by measuring heart rate in response to a submaximal exercise test on a friction-braked cycle ergometer, and calculated by standard protocols. Sex-specific quintiles were formed. Following Blair et al.[7], study members in the lowest quintile were considered to have this health risk.	20%,	18%
Pulmonary function	Pulmonary function was assessed using a computerized spirometer and body plethysmograph[8]. Measurements of vital capacity were repeated to obtain at least three repeatable values (within 5%) followed by full-forced expiratory maneuvers to record the forced expiratory volume in 1s (FEV1): The post-bronchodilator FEV1/FVC ratio after 200 mg salbutamol is reported as the primary lung function measure because it is the most sensitive measure for assessing airway remodeling in a large cohort. Study members with an FEV1/FVC ratio below .70 were classified as having significant airflow limitation[9].	10%,	4%

Periodontal disease	Examinations were conducted in all 4 quadrants using calibrated dental examiners; three sites (mesiobuccal, buccal, and distolingual) per tooth were examined, and gingival recession (the distance in millimeters from the cementoenamel junction to the gingival margin) and probing depth (the distance from the probe tip to the gingival margin) were recorded using a PCP-2 probe. The combined attachment loss (CAL) for each site was computed by summing gingival recession and probing depth (third molars were not included). We report the presence of periodontal disease, defined as 1+ site(s) with 5 or more mm of combined attachment loss.	26%,	17%
Systemic inflammation	Elevation in inflammation was assessed by assaying high-sensitivity C-Reactive Protein (hsCRP, mg/L). High-sensitivity C-reactive protein level is thought to be one of the most reliable measured indicators of vascular inflammation and has been recently endorsed as an adjunct to traditional risk factor screening for cardiovascular risk. hsCRP was measured on a Hitachi 917 analyzer (Roche Diagnostics, GmbH, D-68298, Mannheim, Germany) using a particle enhanced immunoturbidimetric assay. The CDC/AHA definition of high cardiovascular risk (hsCRP >3 mg/L) was adopted to identify our risk group.	15%,	26%

(1) Based on the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) http://www.nhlbi.nih.gov/guidelines/cholesterol/index.htm
(2) Based on the NGSP clinical advisory committee 2010 recommendation http://www.ngsp.org/cac2010.as

Supplementary Table S2: Pearson correlations and multivariate linear regression analyses of internalizing disorder from 11 to 38 years, predicting LTL at 38 years, controlling for physical health indicators at age 38 years. Results are presented for men only (N=419).

	Bivariate Pearson correlation		**Association between internalizing disorder 11-38 and LTL at age 38 years**	
	Internalizing disorder age 11 to 38 years	**LTL at age 38 years**	**β (95% CI)**	**p value**
Internalizing disorder from age 11 to 38 years	-	-	-.137 (-.232, -.042)	**.005**
Controlling for health index variables:				
Obesity	**.111***	.000	-.139 (-.235, -.043)	**.005**
HDL levels	.034	.018	-.138 (-.233, -.042)	**.005**
Triglyceride level	.019	-.094 (p=.055)	-.135 (-.230, -.040)	**.005**
Blood pressure	-.006	**-.173****	-.138 (-.232, -.044)	**.004**
Glycated hemoglobin	**.112***	**-.100***	-.127 (-.223, -.032)	**.009**
Cardiorespiratory fitness	**.098***	-.069	-.131 (-.227, -.036)	**.007**
Pulmonary function	-.003	-.026	-.137 (-.232, -.041)	**.005**
Periodontal disease	**.105***	-.040	-.134 (-.230, -.038)	**.006**
Systemic inflammation	**.128****	-.011	-.138 (-.234, -.041)	**.005**
Physical health at age 38 years	**.129****	**-.135****	-.121 (-.217, -.026)	**.013**

Significant p values are highlighted in boldface; *p < .05; **p < .005.

Supplementary Table S3: Pearson correlations and multivariate linear regression analyses of internalizing disorder from 11 to 38 years, predicting LTL at 38 years, controlling for white blood cell **(**WBC) counts. Results are presented for men only (N=419).

	Bivariate Pearson correlation		**Association between internalizing disorder 11-38 and LTL at age 38 years**	
	Internalizing disorder age 11 to 38 years	**LTL at age 38 years**	**β (95% CI)**	**p value**
Internalizing disorder from age 11 to 38 years	**-**	**-**	-.137 (-.232, -.042)	**.005**
			Controlling for WBC counts:	
Neutrophil	.045	-.010	-.137 (-.232, -.041)	**.005**
Lymphocyte	**.146****	-.041	-.134 (-.230, -.037)	**.007**
Monocyte	.043	.025	-.138 (-.234, -.043)	**.005**
Eosinophil	.051	-.022	-.136 (-.232, -.040)	**.005**
Basophil	.075	**-.108***	-.129 (-.225, -.034)	**.008**
Agranulocyte[1]	.**141****	-.030	-.135 (-.232, -.039)	**.006**
Granulocyte[1]	.055	-.018	-.136 (-.232, -.041)	**.005**
All WBCs	**.099***	-.023	-.136 (-.232, -.040)	**.006**

[1]Agranulocyte (lymphocyte and monocyte); Granulocyte (neutrophil, eosinophil and basophil).
Significant p values are highlighted in boldface; *****p < .05; ******p < .005.

Supplementary Figure S1: Plate template for telomere and single-copy assays. Numbers denote examples of individual samples, each assayed in triplicate. The DNA from the same individual at different age-waves is assayed on the same plate, as shown by the presence of, for example, sample 1 six times within the plate. SDx denotes the standard reference sample serially diluted x-times. NEG denotes negative controls; wells with no DNA included which serve to check for background contamination.

SD1	SD1	SD1	SD2	SD2	SD2	SD3	SD3	SD3	SD4	SD4	SD4	SD5	SD5	SD5	SD6	SD6	SD6	SD7	SD7	SD7	SD8	SD8	SD8
1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6	7	7	7	8	8	8
9	9	9	10	10	10	11	11	11	12	12	12	13	13	13	14	14	14	15	15	15	16	16	16
17	17	17	18	18	18	19	19	19	20	20	20	21	21	21	22	22	22	23	23	23	24	24	24
25	25	25	26	26	26	27	27	27	28	28	28	29	29	29	30	30	30	31	31	31	32	32	32
33	33	33	34	34	34	35	35	35	36	36	36	37	37	37	38	38	38	39	39	39	40	40	40
41	41	41	42	42	42	43	43	43	44	44	44	45	45	45	46	46	46	47	47	47	48	48	48
49	49	49	50	50	50	51	51	51	52	52	52	53	53	53	54	54	54	55	55	55	56	56	56
1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6	7	7	7	8	8	8
9	9	9	10	10	10	11	11	11	12	12	12	13	13	13	14	14	14	15	15	15	16	16	16
17	17	17	18	18	18	19	19	19	20	20	20	21	21	21	22	22	22	23	23	23	24	24	24
25	25	25	26	26	26	27	27	27	28	28	28	29	29	29	30	30	30	31	31	31	32	32	32
33	33	33	34	34	34	35	35	35	36	36	36	37	37	37	38	38	38	39	39	39	40	40	40
41	41	41	42	42	42	43	43	43	44	44	44	45	45	45	46	46	46	47	47	47	48	48	48
49	49	49	50	50	50	51	51	51	52	52	52	53	53	53	54	54	54	55	55	55	56	56	56
NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG

Age 38

Age 26

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