Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis

Web only appendix

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Supplementary Table 1 | Search strategy.

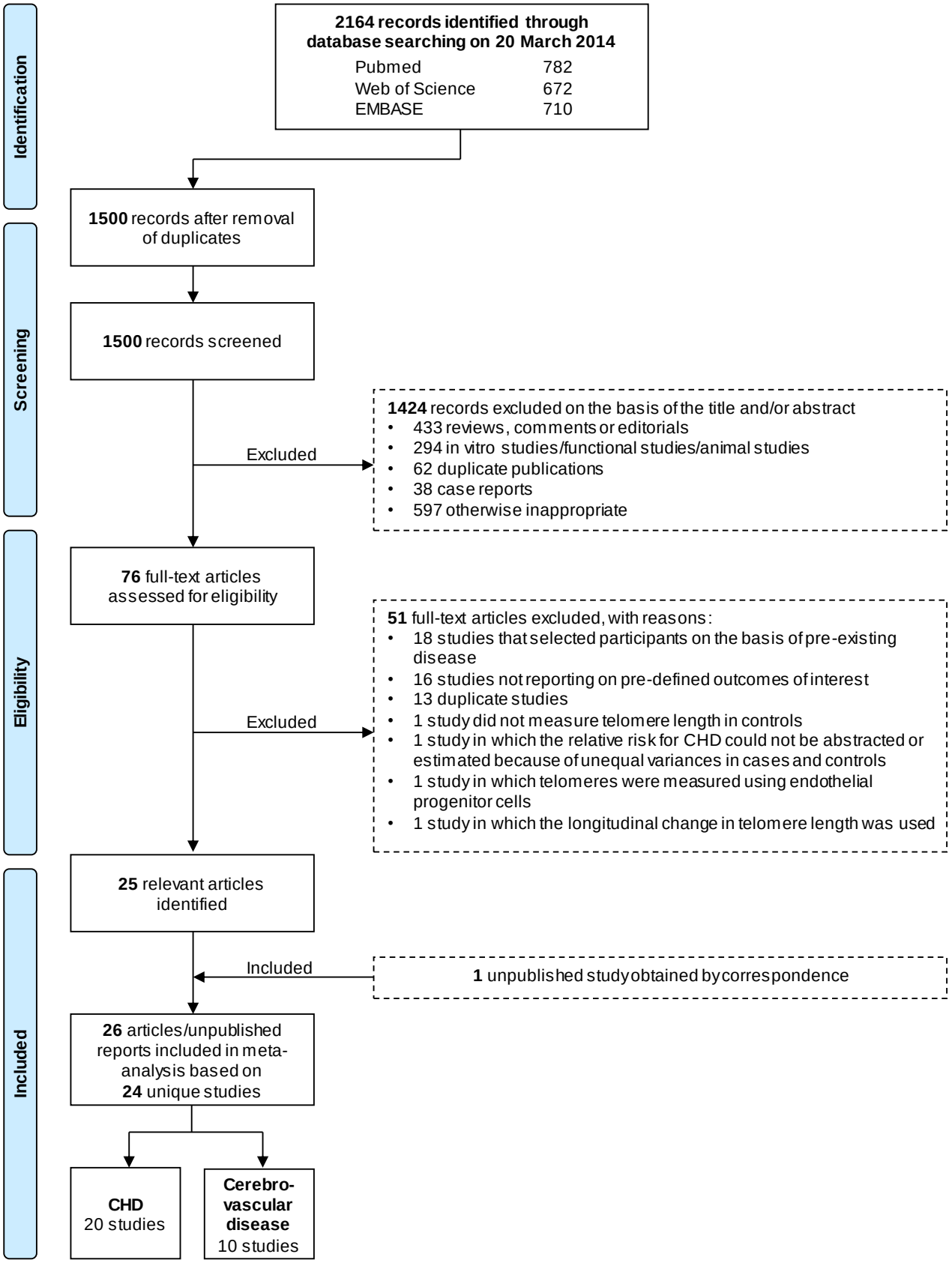
PubMed, search through 20 March 2014
("Telomere"[Mesh] OR "Telomere" OR "Telomeres" OR "telomeric" OR "T/S ratio" OR "T/C ratio") **AND** ("Cardiovascular Diseases"[Mesh] OR "Cardiovascular Diseases" OR "Cardiovascular Disease" OR "Vascular Diseases" OR "Vascular Disease" OR "Ischemic Heart Disease" OR "Myocardial ischaemia" OR "Myocardial ischemia" OR "Acute coronary syndrome" OR "Coronary disease" OR "Coronary heart disease" OR "Coronary artery disease" OR "Coronary occlusion" OR "Coronary stenosis" OR "Coronary artery stenosis" OR "Coronary thrombosis" OR "Myocardial infarction" OR "Heart attack" OR "Cerebrovascular disease" OR "Cerebrovascular diseases" OR "Stroke" OR "Apoplexy" OR "Brain vascular accident" OR "Cerebrovascular accident" OR "Brain ischaemia" OR "Brain ischemia" OR "Ischaemic encephalopathy" OR "Ischemic encephalopathy" OR "Intima media thickness")
Web of Science, search through 20 March 2014
TS=("Telomere" OR "Telomeres" OR "telomeric" OR "T/S ratio" OR "T/C ratio") **AND** TS=("Cardiovascular Diseases" OR "Cardiovascular Disease" OR "Vascular Diseases" OR "Vascular Disease" OR "Ischemic Heart Disease" OR "Myocardial ischaemia" OR "Myocardial ischemia" OR "Acute coronary syndrome" OR "Coronary disease" OR "Coronary heart disease" OR "Coronary artery disease" OR "Coronary occlusion" OR "Coronary stenosis" OR "Coronary artery stenosis" OR "Coronary thrombosis" OR "Myocardial infarction" OR "Heart attack" OR "Cerebrovascular disease" OR "Cerebrovascular diseases" OR "Stroke" OR "Apoplexy" OR "Brain vascular accident" OR "Cerebrovascular accident" OR "Brain ischaemia" OR "Brain ischemia" OR "Ischaemic encephalopathy" OR "Ischemic encephalopathy" OR "Intima media thickness")
EMBASE, search through 20 March 2014
("Telomere" OR "Telomeres" OR "telomeric" OR "T/S ratio" OR "T/C ratio").af **AND** ("Cardiovascular Diseases" OR "Cardiovascular Disease" OR "Vascular Diseases" OR "Vascular Disease" OR "Ischemic Heart Disease" OR "Myocardial ischaemia" OR "Myocardial ischemia" OR "Acute coronary syndrome" OR "Coronary disease" OR "Coronary heart disease" OR "Coronary artery disease" OR "Coronary occlusion" OR "Coronary stenosis" OR "Coronary artery stenosis" OR "Coronary thrombosis" OR "Myocardial infarction" OR "Heart attack" OR "Cerebrovascular disease" OR "Cerebrovascular diseases" OR "Stroke" OR "Apoplexy" OR "Brain vascular accident" OR "Cerebrovascular accident" OR "Brain ischaemia" OR "Brain ischemia" OR "Ischaemic encephalopathy" OR "Ischemic encephalopathy" OR "Intima media thickness").af

Supplementary Table 2 | Further characteristics of the 24 vascular disease studies included in the review

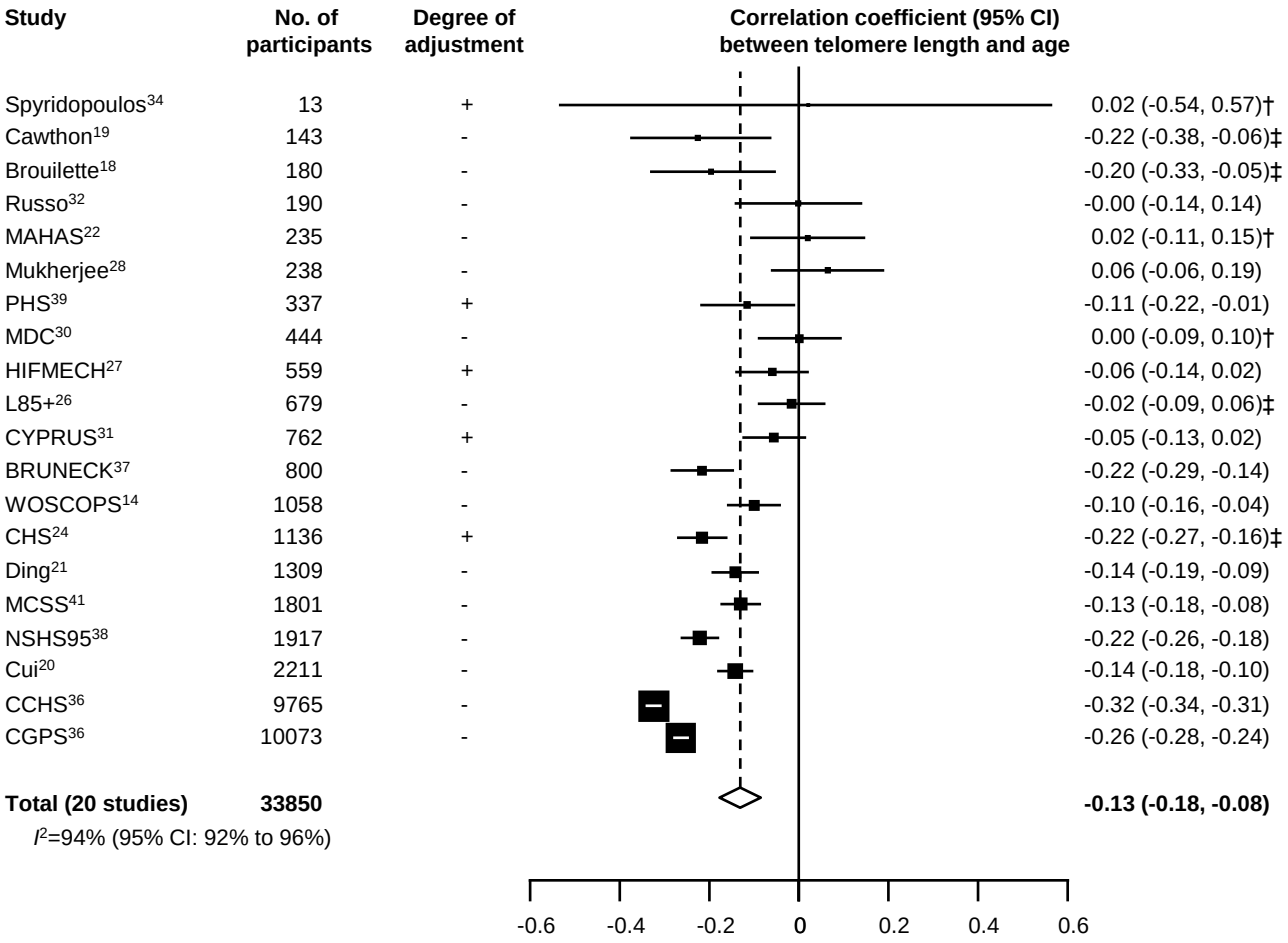
Study / First author	RR (95% CI) as reported by study	Scale of RR reported by study	Conversion factor*	RR (95% CI) in shortest versus longest third of telomere length	Adjustment for confounders†	Mean (SD) cases	Mean (SD) controls	Telomere units	Assay CV (%)	DNA extraction method	No. of experimental replicates
Coronary heart disease studies											
Brouilette[18]	2.79 (1.53 to 5.11)	shortest versus longest fourth	0.86	2.41 (1.44 to 4.05)	++	6.75 (2.55)	7.05 (2.07)	kbp	3.3	salting out	NR
BRUNECK[37]	NR	NR	NA	3.52 (1.29 to 9.57)§	++++	0.05 (0.43)	0.27 (0.52)	T/S	2.4	salting out	4
Cawthon[19]	3.18 (1.36 to 7.45)	bottom versus top half	1.37	4.86 (1.52 to 15.57)	+	NR	NR	NR	5.8	PC	3
CCHS[36]	1.08 (0.99 to 1.18)	per 1000 bp (1.15 SD) decrease	1.87	1.16 (0.98 to 1.36)	++++	NR	NR	NR	9	QiaAMP	4
CGPS[36]	1.31 (1.02 to 1.67)	per 1000 bp (1.48 SD) decrease	1.45	1.48 (1.03 to 2.11)	++++	NR	NR	NR	9	QiaAMP	4
CHS[23]	1.55 (0.85 to 2.83)	per 1000 bp (2.5 SD) decrease	0.87	1.47 (0.87 to 2.48)	++	NR	NR	NR	1.5	salting out	NR
Cui[20]	1.13 (1.02 to 1.34)	per SD decrease	2.18	1.31 (1.04 to 1.89)	+++	-0.2 (0.45)	-0.03 (0.51)	$\log_e$ T/S	1.28	QG-Mini80; DB-S kit	3
CYPRUS[31]	0.60 (0.36 to 1.12)	per $\log_e$ T/S unit (1.64 SD) increase	-1.33	1.98 (0.86 to 3.91)	+++	-1.35 (0.67)	-1.11 (0.61)	$\log_e$ T/S	5.6	QiaAMP	2
HABC[29]	1.00 (0.90 to 1.10)	per 1000 bp (0.83 SD) decrease	2.63	1 (0.76 to 1.29)	++	4.8 (1.3)	4.9 (1.2)	kbp	5.8	NR	3
HIFMECH[27]	NR	NR	NA	1.37 (1 to 1.89)§	++	7.85 (4.01)	8.04 (4.46)	$\log_e$ T/S	5.6	salting out	2
L85+[26]	1 (0.9 to 1.1)	shortest versus longest third	1.00	2.23 (1.06 to 4.66)	+	NR	NR	NR	5.12	QiaAMP	4
MAHAS[22]	NR	NR	NA	1.09 (0.47 to 2.54)‡§	-	1.12 (0.24)§	1.13 (0.24)§	T/S	5.8	salting out	2
MDC[30]	NR	NR	NA	1 (0.71 to 1.42)‡§	+	0.65 (0.19)§	0.65 (0.2)§	T/S	NR	QiaAMP	3
Mukherjee[28]	NR	NR	NA	2.15 (1.23 to 3.78)‡	-	1.18 (0.45)	1.34 (0.44)	T/S	NR	salting out	2
NSHS95[38]	1.25 (0.82 to 1.9)	shortest versus longest third	1.00	1.25 (0.82 to 1.9)	++++	NR	NR	NR	6.50	NR	6
PHS[39]	1.62 (1.14 to 2.3)	per $\log_e$ T/S unit (1.28 SD) decrease	1.55	2.11 (1.22 to 3.64)	+++	3.41 (0.63)	3.52 (0.78)	$\log_e$ T/S	4.5	QiaAMP	2
Russo[32]	NR	NR	NA	0.89 (0.58 to 1.38)‡	+	0.77 (0.19)	0.76 (0.19)	normalized T/S	NR	salting out	3
SMS[35]	NR	NR	NA	4.78 (2.2 to 10.37) ‡§	+	5.67 (2.14)§	6.96 (1.72)§	kbp	NR	NR	NR
Spyridopoulos[34]	NR	NR	NA	5.8 (0.71 to 47.19)§	+	6.54 (0.55)§	6.96 (0.86)§	kbp	5.5	NA	NR
WOSCOPS[14]	1.95 (1.33 to 2.84)	shortest versus longest third	1.00	1.95 (1.33 to 2.84)	++	NR	NR	NR	NR	salting out	2
Cerebrovascular disease studies											
BRUNECK[37]	NR	NR	NA	2.35 (0.97 to 5.68)§	++++	0.02 (0.44)	0.28 (0.51)	T/S	2.4	salting out	4
Cawthon[19]	1.35 (0.36 to 5.13)	shortest versus longest fourth	0.86	1.29 (0.42 to 4.07)	+	NR	NR	NR	5.8	PC	3
CHS[24]	1.27 (0.34 to 4.74)	shortest versus longest fourth	0.86	1.23 (0.4 to 3.8)	++	NR	NR	NR	1.7	salting out	NR
Ding[21]	2.12 (1.62 to 2.77)	shortest versus longest fourth	0.86	1.91 (1.51 to 2.4)	+++	NR	NR	NR	1.3	QG-Mini80; DB-S kit	NR
HABC[29]	1.00 (0.80 to 1.20)	per 1000 bp (0.83 SD) decrease	2.61	1 (0.56 to 1.61)	++	4.8 (1.1)	4.9 (1.2)	kbp	5.8	NR	3
Jiang[25]	4.00 (1.28 to 12.45)	bottom versus top half	1.37	6.64 (1.41 to 31.41)	++	0.92 (0.77)	1.68 (1.24)	log T/S	6.70	QiaAMP	NR
MCSS[41]	1.37 (1.06 to 1.77)	shortest versus longest third	1.00	1.37 (1.06 to 1.77)	+++	-0.89 (0.98)	-0.63 (0.98)	$\log_e$ T/S	NR	salting out	3
NHS[33]	0.98 (0.54 to 1.79)	shortest versus longest fourth	0.86	0.98 (0.59 to 1.65)	++++	NR	NR	NR	22	QiaAMP	NR
PHS[40]	1.1 (0.51 to 2.39)	per $\log_e$ T/S unit (1.79 SD) decrease	1.21	1.12 (0.44 to 2.87)	+++	3.83 (0.55)	3.83 (0.56)	$\log_e$ T/S	4.5	QiaAMP	2
SMS‖	NR	NR	NA	0.86 (0.24 to 3.1)‡§	+++	6.72 (2.29)§	6.48 (3.65)§	kbp	NR	NR	NR

Abbreviations: CI, confidence interval; **CV**, coefficient of variation; **kbp**, kilobase-pairs; **NA**, not applicable; **NR**, not reported; **PC** – phenol-chloroform; **RR**, relative risk; **SD**, standard deviation. *To convert reported log RR to log RR in shortest versus longest third of the telomere length distribution; †-no adjustment, + adjusted for age and/or sex, ++ age, sex, and non-lipid risk factors, +++ adjusted for age, sex, smoking, body mass index, diabetes, blood pressure and lipid markers, ++++adjusted for preceding plus C-reactive protein, and physical activity; ‡calculated from the mean difference in telomere length between cases and controls; §obtained through correspondence; ‖previously unpublished. **Study acronyms: CCHS**, Copenhagen City Heart Study; **CGPS**, Copenhagen General Population Study; **CHS**, Cardiovascular Health Study; **HABC**, Health Aging & Body Composition Study; **HIFMECH**, Hypercoagulability and Impaired Fibrinolytic function MECHanisms predisposing to myocardial infarction; **L85+**, Leiden 85-plus Study; **MAHAS**, MacArthur Health Aging Study; **MCSS,** Multicenter Chinese Stroke Study; **MDC**, Malmö Diet and Cancer Study; **NHS**, Nurses' Health Study; **NSHS95,** 1995 Nova Scotia Health Survey; **PHS**, Physicians' Health Study; **SMS**, Scottish Mental Survey; **WOSCOPS**, West of Scotland Coronary Prevention Study.

Supplementary Figure 1 | Study flow diagram.

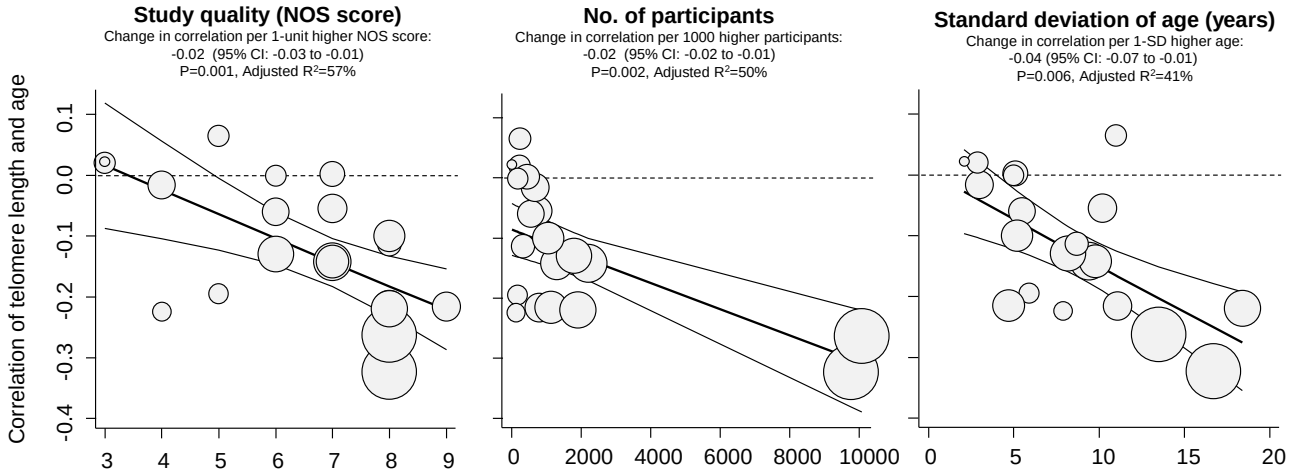


Supplementary Figure 2 | Reported correlation coefficients between telomere length and age in 20 studies*.



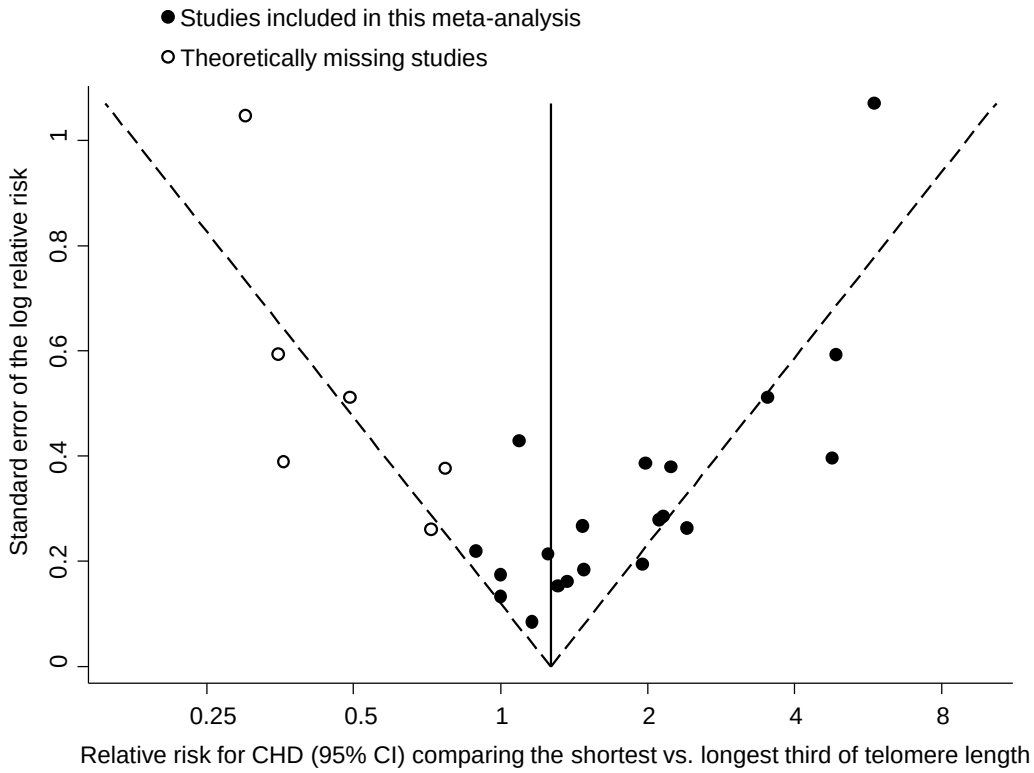
*20/24 studies reported correlation coefficients between telomere length and age using participants from cohort studies or controls from case-control studies. Sizes of data markers are proportional to the inverse of the within-study variance. The overall correlation was calculated by random effects meta-analysis. CI, confidence interval. Degree of adjustment: - unadjusted, + adjusted for sex. †obtained through correspondence; ‡estimated from the t-statistic of the linear association between telomere length and age.

Supplementary Figure 3 | Correlation between telomere length and age as a function of selected characteristics.



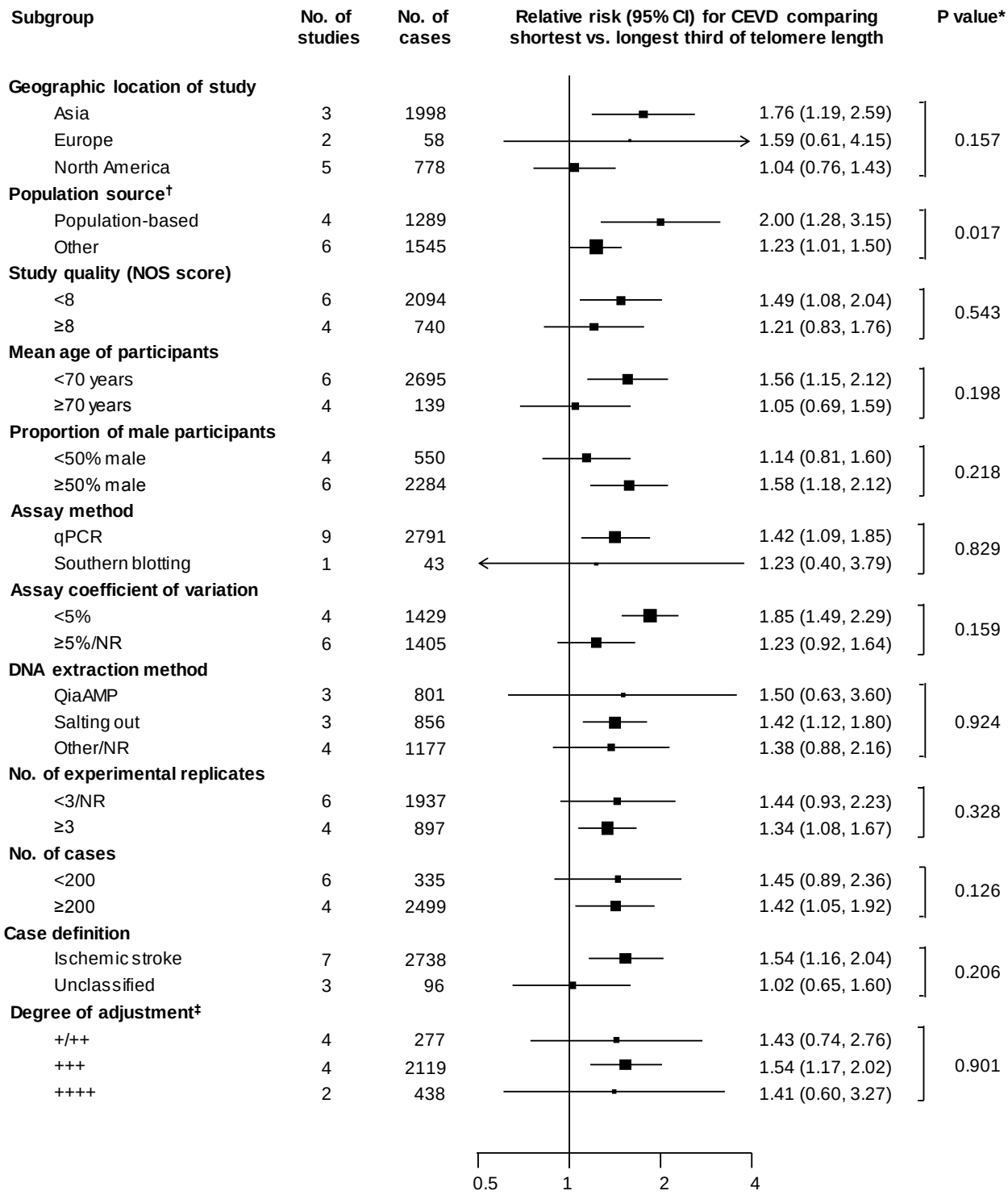
The plotted data show how the strength of the relationship between telomere length and age varies by the selected characteristic. The plotted solid lines indicate the predicted correlation and its 95% confidence interval. Circle size is proportional to the inverse of the within-study variance. CI – confidence interval, NOS, Newcastle-Ottawa Scale, SD – standard deviation. Study quality, the no. of participants, and standard deviation of age collectively explained 80% of the between-study heterogeneity in reported correlations between telomere length and age. The correlation between telomere length and age was: -0.22 (95% CI: -0.27 to -0.16) in studies with a NOS score ≥8; -0.20 (95% CI: -0.25 to -0.14) in studies with >1,000 participants; and -0.16 (95% CI: -0.22 to -0.10) in studies with an standard deviation of age ≥8 years.

Supplementary Figure 4 | Funnel plot of study-specific CHD relative risks.



The P value from Egger´s regression asymmetry test for publication bias was 0.0006. The overall relative risk for CHD estimated by random effects meta-analysis was 1.54 (95% CI: 1.30 to 1.83) in the shortest versus longest third of telomere length. The corresponding relative risk estimated by Duval´s nonparametric 'tim and fill' method was 1.34 (95% CI: 1.12 to 1.60). The plotted lines correspond to the summary fixed effect with pseudo-95% confidence intervals. CI – confidence interval, CHD – coronary heart disease.

Supplementary Figure 5 | Telomere length and cerebrovascular disease risk grouped by recorded study level characteristics.



The sizes of the data markers are proportional to the inverse of the variance of the relative risk. CEVD – cerebrovascular disease; CI, confidence interval; NR, not reported; NOS, Newcastle-Ottawa Scale. *P-values for heterogeneity from meta-regression; studies in which the characteristic was not reported were not included in the calculation of the P-value; for continuous characteristics, the P-value reflects the linear test of association. †Population source of cohort or controls in case-control studies; ‡degree of adjustment: +/++adjusted for age or age, sex & non-lipid risk factors, +++adjusted for age, sex, body mass index, diabetes, smoking, blood pressure & lipid-markers, ++++adjusted for preceding plus C-reactive protein and physical activity