Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study

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Summary

Background Inter-individual differences in biological ageing could affect susceptibility to coronary heart disease. Our aim was to determine whether mean leucocyte telomere length is a predictor of the development of coronary heart disease.

Methods We compared telomere lengths at recruitment in 484 individuals in the West of Scotland Primary Prevention Study (WOSCOPS) who went on to develop coronary heart disease events with those from 1058 matched controls who remained event free. We also investigated whether there was any association between telomere length and observed clinical benefit of statin treatment in WOSCOPS.

Findings Mean telomere length decreased with age by 9% per decade (95% CI 3·6–14·1; p=0·001) in controls; much the same trend was seen in cases (–5·9% per decade, –3·1 to 14·1; p=0·1902). Individuals in the middle and the lowest tertiles of telomere length were more at risk of developing a coronary heart disease event than were individuals in the highest tertile (odds ratio [OR] for coronary heart disease: 1·51, 95% CI 1·15–1·98; p=0·0029 in the middle tertile; 1·44, 1·10–1·90, p=0·0090 in the lowest). In placebo-treated patients, the risk of coronary heart disease was almost double in those in the lower two tertiles of telomere length compared with those in the highest tertile (1·93, 1·33–2·80, p=0·0005 in the middle tertile; 1·94, 1·33–2·84, p=0·0006 in the lowest). By contrast, in patients treated with pravastatin, the increased risk with shorter telomeres was substantially attenuated (1·12, 0·75–1·69, p=0·5755 in the middle tertile; 1·02, 0·68–1·52, p=0·9380 in the lowest).

Interpretation Mean leucocyte telomere length is a predictor of future coronary heart disease events in middle-aged, high-risk men and could identify individuals who would benefit most from statin treatment. Our findings lend support to the hypothesis that differences in biological ageing might contribute to the risk—and variability in age of onset—of coronary heart disease.

Lancet 2007; 369: 107–14

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Introduction

Although epidemiological studies have identified several cardiovascular risk factors that potentially explain most of the risk of coronary heart disease in a population,[1] at an individual level there is wide variation in both the occurrence of coronary heart disease and age of manifestation, even in individuals with much the same risk factor profiles. The reasons for this wide inter-individual variation in susceptibility are poorly understood. Coronary heart disease is an age-associated disease. Histologically, cellular senescence is a major feature of atherosclerotic plaques,[2,3] and in-vitro induction of senescence in coronary endothelial cells causes the expression of molecules implicated in atherogenesis.[4] Therefore, the hypothesis has emerged that, at least to some extent, inter-individual variation in risk of coronary heart disease might result from variation in the rate of biological ageing.[5–7]

Telomeres are the extreme ends of chromosomal DNA, made up of a large number of tandem repeats of the sequence TTAGGG. Although the full extent of their functions is not fully understood, they are involved in the maintenance of cellular stability.[8] Because DNA polymerase cannot fully complete the replication of the 3' end of linear DNA, telomeres progressively shorten with repeated cell division.[9] Olovnikov[10] was the first to suggest that this shortening is a potential mechanism for a biological clock that determines cellular behaviour, and this concept has since been lent support by experiments.[11,12] In many cell types, senescence and subsequent cell death often occurs when the mean telomere length reaches a critical value.[13] Therefore, mean telomere length provides a marker for biological age, at least at the cellular level, with shorter telomeres indicating increased biological age.

We have shown that mean telomere length in leucocytes is shorter in patients with severe triple vessel coronary artery disease than it is in individuals with angiographically normal coronary arteries,[5] and also in patients who have had a premature myocardial infarction before age 50 years than in age and sex matched people without such a history.[6] We have also shown that the association of shorter telomeres with coronary heart disease is independent of classic and novel risk factors for coronary heart disease, including markers of inflammation. A limitation of these cross-sectional studies is that they

cannot exclude the possibility that the shorter telomere lengths might simply be a consequence of the development of coronary heart disease, rather than being a primary abnormality. Here, by use of the DNA bank established as part of the West of Scotland Primary Prevention Study (WOSCOPS)[14] and a recently described PCR-based assay for measuring telomere length,[15] we investigated the prospective association of leucocyte telomere length with development of coronary heart disease. Because WOSCOPS randomly assigned some patients to receive statin treatment,[14] we also investigated whether there was any relation between mean telomere length and the observed benefit of statin treatment.

Methods

Patients

In WOSCOPS, 6595 statin-naive men aged 45–64 years (mean 55·2 years) who had a total cholesterol concentration of 6·5–7·8 mmol/L at recruitment, an LDL-cholesterol concentration over 4·5 mmol/L on a National Cholesterol Education Programme (NCEP) diet on one occasion before randomisation, and no history of a myocardial infarction were randomly assigned to receive pravastatin (40 mg daily) or placebo.[14] The study complied with the Declaration of Helsinki, was approved by the local ethics committee, and participants gave written informed consent. Over a mean follow-up period of 4·9 years, 503 men experienced the primary endpoint (a composite of non-fatal myocardial infarction and death from coronary heart disease), and 77 individuals had a coronary revascularisation procedure. Pravastatin reduced the risk of the primary endpoint by 31%.

For the purpose of nested case-control studies in WOSCOPS, individuals with a primary endpoint or who underwent coronary revascularisation (cases) were matched on the basis of age (with 2-year age categories), duration of follow-up, and smoking status, as previously described[16] with two individuals (controls) who remained event-free during the study. Leucocyte DNA, extracted from blood obtained at recruitment and suitable for telomere length analysis, was available for 484 cases and 1058 controls. The demographic characteristics of individuals with missing samples were much the same as those studied (data not shown).

	Cases (n=484)	Controls (n=1058)	Odds ratio	p
Age* (years)	56·9 (5·1)	56·7 (5·2)	..	..
Smokers*	259 (54%)	585 (55%)	..	..
Body mass index (kg/m^2)	26·0 (3·1)	25·6 (3·2)	1·08 (1·01–1·15)	0·0269
Systolic blood pressure (mm Hg)	139·5 (17·2)	135·6 (17·1)	1·14 (1·07–1·21)	<0·0001
Diastolic blood pressure (mm Hg)	85·5 (10·4)	83·6 (10·2)	1·18 (1·07–1·32)	0·0016
LDL cholesterol (mmol/L)	5·02 (0·46)	4·95 (0·44)	1·43 (1·13–1·82)	0·0031
HDL cholesterol (mmol/L)	1·07 (0·22)	1·14 (0·25)	0·67 (0·63–0·71)	<0·0001
Triglycerides (mmol/L)	1·95 (0·82)	1·84 (0·77)	1·11 (1·03–1·20)	0·0064
Diabetes	9 (2%)	13 (1%)	1·51 (0·64–3·56)	0·3451
Hypertension	112 (23%)	162 (15%)	1·65 (1·26–2·17)	0·0003
CRP (mg/L)	2·41 (2·79)	1·88 (2·93)	1·28 (1·14–1·43)	<0·0001
Fibrinogen (mmol/L)	4·52 (0·87)	4·35 (0·86)	1·27 (1·12–1·45)	0·0002
White blood cell count (×10^6 cells per mL)	7·08 (1·90)	6·76 (1·87)	1·13 (1·06–1·20)	0·0002
Glucose (mmol/L)	4·87 (0·73)	4·78 (0·69)	1·18 (1·02–1·37)	0·0268
Alcohol consumption (units per week)	10·8 (13·2)	11·2 (12·7)	1·00 (0·99–1·01)	0·5840
Statin treatment	195 (40%)	541 (51%)	0·65 (0·52–0·80)	<0·0001

Data are n (%) or mean (SD). p values are from univariate conditional logistic regression analyses. For CRP, the geometric mean is given as the distributions were skewed and the p value is based on analysis of $\log_{10}$ CRP. For qualitative variables, the odds ratios and 95% CI are for the presence of the variable. For quantitative variables, the values are for the following changes: 2 kg/m^2 higher body-mass index, 10 mm Hg higher systolic or diastolic blood pressure, 0·25 mmol/L higher HDL-cholesterol concentration, 0·57 mmol/L higher triglyceride level, and a unit change in the other variables. *Age and smoking were matching criteria.

Table 1: **Characteristics of cases and controls at recruitment**

Procedures

The individuals enrolled in WOSCOPS were extensively assessed for major cardiovascular risk factors during recruitment, as described previously.[17] Lipid measurements included fasting total cholesterol, LDL cholesterol, HDL cholesterol, and trigyceride concentrations. Haematological variables, including the white blood cell count, were determined, and fibrinogen was assayed by heat-precipitation nephelometry. C-reactive protein (CRP) was measured in plasma obtained at recruitment and stored at –70°C by use of a high-sensitivity, two-site ELISA.[16]

Leucocyte telomere length was measured with a quantitative PCR-based technique that compares telomere repeat sequence copy number to single-copy gene (36b4) copy number in a given sample.[15] Duplicate DNA samples were amplified in parallel 20 µL PCR reactions; both reactions included 20 mmol/L Tris hydrochloride buffer (pH 8·4), 50 mmol/L potassium chloride, 200 µmol/L each of deoxyadenosine triphosphate, deoxythymidine triphosphate, deoxycytidine triphosphate, and deoxyguanine triphosphate, 1% dimethyl sulfoxide, 2·5 mmol/L dithiothreitol, 0·4×Sybr Green I (Invitrogen, Paisley, UK), and 20 ng of sample DNA. The telomere-specific reaction included 1·5 mmol/L magnesium chloride, 0·8 units of Platinum Taq DNA polymerase (Invitrogen, Paisley, UK), and 300 nmol/L of telomere-specific primers (forward: 5′CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT3′; reverse: 5′GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT3′). The 36b4 reaction included 3·5 mmol/L magnesium chloride, 0·5 units of Platinum Taq, and 300 nmol/L of the forward primer (5′CAGCAAGTGGGAAGGTGTAATCC3′) primer and 500 nM of the reverse primer (5′CCCATTCTATCATCAACGGGTACAA3′). All PCRs were done with the Corbett Research Rotor-Gene 3000 Real-time Thermal Cycler (Corbett Research, Cambridge, UK). The thermal cycling profile for both amplicons began with a 95°C incubation for 10 min to activate the Platinum Taq DNA polymerase. For the telomere PCR, there followed 25 cycles of 15 s at 95°C and 1 min at 58°C. For the 36b4 PCR, there followed 35 cycles of 15 s at 95°C and 1 min at 58°C. The specificity of all

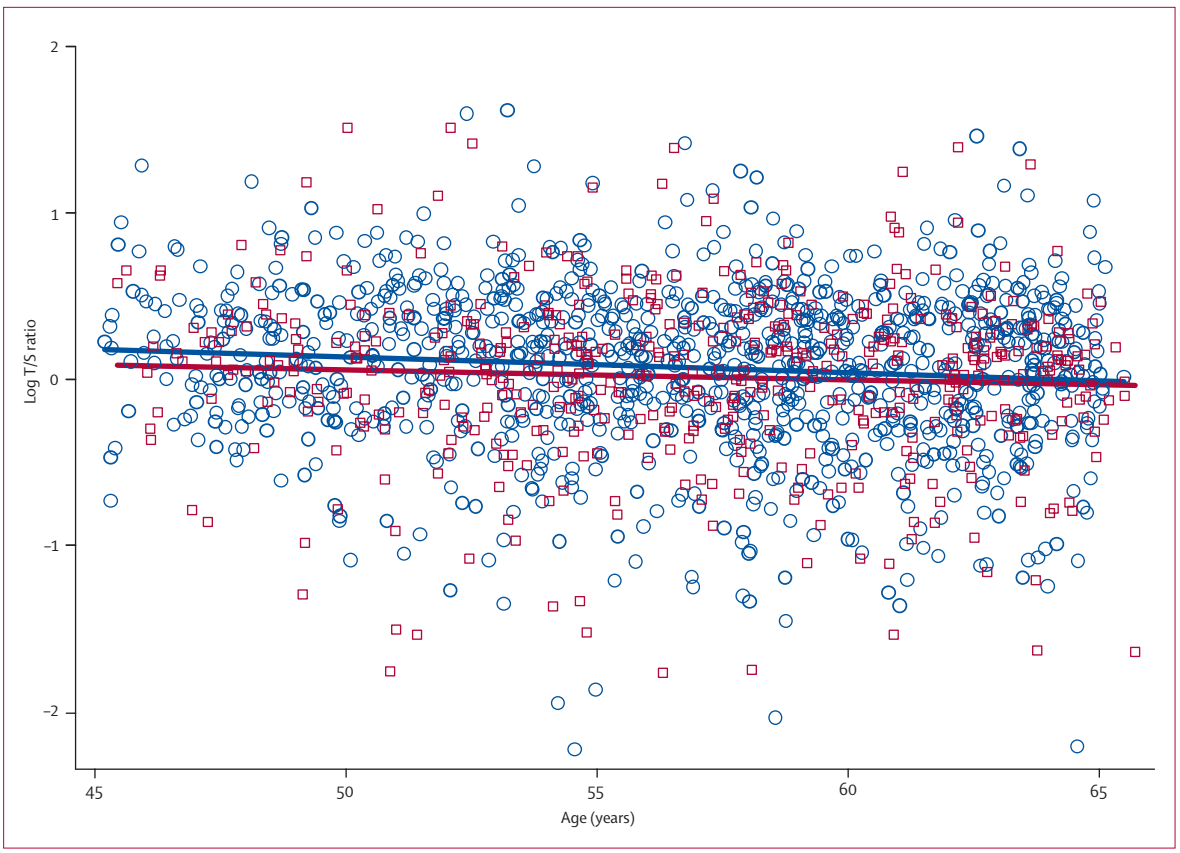

Figure 1: **Telomere length as a function of age in controls and cases**
Controls are shown as blue circles (n=1058), cases as red squares (n=484). Telomere length is plotted as log T/S ratio. *r* values were 0·10 (p=0·001) for controls and 0·06 (p=0·1902) for cases.

amplifications was determined by melting curve analysis. 34 study samples, a calibrator sample, and one no-template control sample (all in duplicate) were processed per run.

During optimisation of the assays, a dilution series (100 ng–1·56 ng DNA, two-fold dilution, seven points) was run for both the telomere and 36b4 PCRs. For both assays, linearity (R^2>0·99) over this range of input DNA was noted. Test samples with threshold cycle numbers that fell outside the range defined by the standard curves were re-run at a different concentration to ensure that they were amplified within the linear range. Linearity of the assays over a similar range of input DNA samples has also been shown in other studies.[15]

The PCR data were analysed with the comparative quantitation approach.[18] Briefly, this approach, implemented with the Corbett Research Rotor-Gene 6.0.1 analysis software, calculates the second derivative of the real-time amplification curve. The peak of the second derivative curve represents the maximum exponential growth of the product. The point 80% back from the peak is defined as the takeoff point, and the amplification efficiency calculated from the section of curve between these two points. This calculation was done for all the samples in a run, and mean amplification efficiency (MAE) calculated. The concentration value for every unknown sample was calculated relative to the calibrator sample with the formula: relative concentration $=MAE^{(\text{calibrator takeoff}-\text{sample takeoff})}$. The same calibrator sample was used in all runs to allow comparison of results across runs. This process was done for both the telomere and single-copy gene reactions, and telomere length expressed as a ratio of the two (telomere/single-copy gene, referred to as the T/S ratio), with the mean data from the duplicate runs. All analyses were done blinded to case-control status of the individual.

To confirm that the PCR assay could identify changes in telomere length, we analysed the ability of the assay to detect the known attrition in mean telomere length that occurs in human vein endothelial cells during in-vitro passage (webappendix 1). We also measured telomere length in 32 randomly chosen DNA samples in duplicate on consecutive days to assess the reproducibility of the assay (webappendix 2). To compare measurements of telomere length obtained with the PCR assay with that determined with conventional Southern blotting, 18 samples from individuals aged 32–54 years were chosen at random from our DNA bank and analysed in triplicate with both mean terminal restriction fragment

See **Online** for webappendices 1 and 2

	Controls				Cases			
	Highest tertile (n=355)	Middle tertile (n=355)	Lowest tertile (n=348)	p	Highest tertile (n=123)	Middle tertile (n=186)	Lowest tertile (n=175)	p
Age (years)	56·2 (5·3)	56·8 (5·3)	57·2 (5·0)	0·0329	56·5 (4·9)	56·9 (5·4)	57·3 (4·9)	0·4013
Smokers	198 (56%)	196 (55%)	191 (55%)	0·9716	66 (54)	104 (56)	89 (51)	0·6287
Body-mass index (kg/m^2)	25·5 (2·9)	25·7 (3·5)	25·6 (3·2)	0·6143	26·3 (3·3)	25·7 (3·2)	26·2 (2·9)	0·1872
SBP (mm Hg)	135·6 (16·7)	135·9 (17·0)	135·5 (17·6)	0·9529	141·0 (14·7)	140·2 (18·5)	137·6 (17·3)	0·1956
DBP (mm Hg)	83·6 (10·2)	83·4 (9·6)	83·9 (10·9)	0·7635	85·0 (9·5)	85·9 (11·2)	85·4 (10·3)	0·7816
LDL cholesterol (mmol/L)	4·95 (0·44)	4·97 (0·45)	4·92 (0·42)	0·2924	5·08 (0·44)	4·96 (0·45)	5·05 (0·48)	0·0468
HDL cholesterol (mmo/L)	1·13 (0·23)	1·16 (0·28)	1·14 (0·25)	0·5633	1·03 (0·20)	1·09 (0·22)	1·08 (0·24)	0·0664
Trigycerides (mmol/L)	1·85 (0·81)	1·85 (0·76)	1·82 (0·75)	0·8474	1·92 (0·79)	1·97 (0·87)	1·96 (0·81)	0·8875
Diabetes	1 (0%)	4 (1%)	8 (2%)	0·0507	3 (2%)	4 (2%)	2 (1%)	0·6686
Hypertension	60 (17%)	57 (16%)	45 (13%)	0·3149	24 (20%)	46 (25%)	42 (24%)	0·5359
C–reactive protein (mg/L)*	2·00 (3·00)	1·79 (2·99)	1·85 (2·79)	0·3926	2·33 (2·84)	2·33 (2·76)	2·55 (2·79)	0·6806
Fibrinogen (mmol/L)	4·38 (0·86)	4·37 (0·90)	4·30 (0·83)	0·3738	4·46 (0·82)	4·51 (0·89)	4·57 (0·90)	0·5686
White blood cell count (×10^{6}cells per mL)	6·90 (1·91)	6·72 (1·77)	6·67 (1·93)	0·2297	7·11 (2·12)	7·16 (1·82)	6·98 (1·81)	0·6531
Glucose (mmol/L)	4·75 (0·59)	4·79 (0·71)	4·80 (0·75)	0·6429	5·01 (0·83)	4·81 (0·70)	4·83 (0·68)	0·0470
Alcohol consumption (units/week)	11·7 (13·3)	10·5 (11·5)	11·5 (13·3)	0·4089	11·2 (13·8)	11·8 (14·2)	9·4 (11·6)	0·2039
Statin treatment	175 (49%)	179 (50%)	187 (54%)	0·4742	61 (50%)	68 (37%)	66 (38%)	0·0502

T/S ratios for tertile cutoffs were <0·909 for lowest tertile, >0·909 to <1·37 for the middle tertile, and >1·37 for highest tertile. Data are n (%) or mean (SD). *Geometric mean; p value based on analysis of $\log_{10}$ CRP.

Table 2: **Distribution of demographic characteristics and cardiovascular risk factors at recruitment by tertiles of mean telomere length in controls and cases**

See Online for webappendix 3

triplicate with both mean terminal restriction fragment analysis and real-time PCR (webappendix 3). Mean terminal restriction fragment analysis was done as previously described.[5]

Statistical analysis

Characteristics of cases and controls were compared with univariate conditional logistic regression analysis. Because the T/S ratios were not normally distributed, the data were log transformed. Limits for the tertiles of telomere length were derived from the control group as follows: T/S ratio greater than 1·37 for the highest tertile, 0·909–1·37 for the middle tertile, and less than 0·909 for the lowest tertile. Based on data shown in webappendix 3, these correspond to telomere lengths greater than 7·13 kb, 6·50–7·13 kb, and less than 6·50 kb, respectively, as measured by Southern blotting. We assessed the effects of age, case-control status, and other individual risk factors on telomere length with regression models. Distribution of demographic and risk factors in control and case individuals in different tertiles of telomere length were compared with χ^2 tests for categorical variables and analysis of variance for quantitative variables. We analysed the relation of telomere length with quantitative variables with Spearman partial correlations adjusted for age in controls and cases separately. We calculated odds ratios (OR) for the risk of coronary heart disease events for the middle and lower tertiles of telomere length compared with the highest tertile with conditional logistic regression that accounted for the matching in the design. The results were repeated by partitioning on pravastatin use, and a test of interaction between tertile length and study treatment was done. All analyses were done with SAS version 9.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the report for publication. The corresponding author had access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Table 1 shows the characteristics of cases and controls at the time of recruitment to WOSCOPS, together with the OR for coronary heart disease risk associated with changes in—or presence of—various risk factors. Cases had higher body-mass index, triglyceride and LDL-cholesterol concentrations, and lower HDL-cholesterol concentrations than did controls. Systolic blood pressure was, on average, 3·9 mm Hg higher in cases than in controls; likewise, diastolic blood pressure was 1·9 mm Hg higher in cases than in controls. Cases reported having a history of hypertension more often than did controls. There was no difference in the prevalence of diabetes, although the fasting plasma glucose level was marginally higher in cases than in controls. As previously reported,[16] white blood cell count, CRP, and fibrinogen levels were all higher in cases than in controls. There was no difference in self-reported weekly alcohol consumption.

Figure 1 shows the distribution of log T/S ratio as a function of chronological age in all individuals. There was a significant fall in T/S ratio with age in controls (–9% per decade, 95% CI –3·6 to –14·1; p=0·001). Much the same

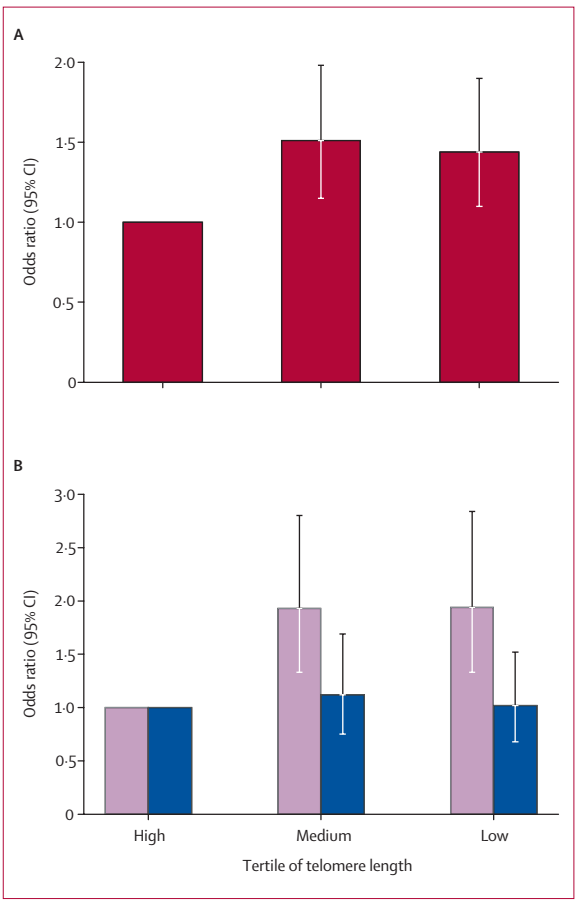

Figure 2: **Risk of clinical event in individuals with shorter telomere length compared with individuals with the longest telomeres**
(A) All individuals, (B) individuals partitioned by pravastatin (blue) or placebo (purple) treatment. OR (95% CI) are shown.

trend was seen in cases, although this was not significant (–5·9% per decade, 3·1 to –14·1; p=0·190). However, there was no difference in the regression line slopes between controls and cases (p=0·53).

Table 2 shows demographic characteristics and cardiovascular risk factors in different tertiles of telomere length in control and case individuals. Correlation coefficients, adjusted for age, between telomere length and the quantitative traits are shown in the webtable. There were no striking differences in any characteristic between individuals in different tertiles, nor were there strong correlations with any of the quantitative traits.

Figure 2A shows the risk of a coronary heart disease event in individuals in different tertiles of T/S ratio at recruitment. Individuals in the middle and lowest tertiles had significantly higher risk than did those in the highest tertile (OR 1·51, 1·15–1·98, p=0·0029 in the middle tertile; 1·44, 1·10–1·90, p=0·0090 in the lowest tertile; table 3).

By calculation of the mean difference between telomere length in cases and controls, and by use of the age regression line in controls, we noted that, on average, mean telomere length in leucocytes at recruitment in individuals who developed coronary heart disease was comparable to control individuals chronologically 6 years older.

When individuals were partitioned by whether they were taking pravastatin or not, a difference in risk associated with shorter telomere length was seen in the two groups (figure 2B and table 3). In the placebo group, risk of coronary heart disease nearly doubled in those in the lower two tertiles compared with individuals in the highest tertile (1·93, 1·33–2·81, p=0·0005 in the middle tertile; 1·95, 1·33–2·84, p=0·0006 in the lowest tertile), whereas in the pravastatin group, the increased risk with shorter telomeres was attenuated by treatment (1·12, 0·75–1·69, p=0·5755 in the middle tertile; 1·02, 0·68–1·53, p=0·9380 in the lowest tertile). Analysis by quintiles showed increased risk in the three lowest quintiles (data not shown). The interaction between T/S ratio and treatment on coronary heart disease risk was significant (p=0·0422).

In those individuals at lowest risk on the basis of telomere length (ie, the highest tertile), there was no apparent benefit from statin treatment (OR 1·03, 0·68–1·55, p=0·8883), whereas in the other tertiles the benefits of treatment were substantial (0·57, 0·39–0·81,

See Online for webtable

	Cases	Controls	OR (95% CI)	p
All individuals				
Highest tertile	123 (25%)	355 (34%)	(ref)	
Middle tertile	186 (38%)	355 (34%)	1·51 (1·15–1·98)	0·0029
Lowest tertile	175 (36%)	348 (33%)	1·44 (1·10–1·90)	0·0090
Individuals who received placebo				
Highest tertile	62 (21%)	180 (35%)	(ref)	
Middle tertile	118 (41%)	176 (34%)	1·93 (1·33–2·81)	0·0005
Lowest tertile	109 (38%)	161 (31%)	1·95 (1·33–2·84)	0·0006
Individuals who received pravastatin				
Highest tertile	61 (31%)	175 (32%)	(ref)	
Middle tertile	68 (35%)	179 (33%)	1·12 (0·75–1·69)	0·5755
Lowest tertile	66 (34%)	187 (35%)	1·02 (0·68–1·53)	0·9380

Data are n (%), unless otherwise indicated. T/S ratios for tertile cutoffs were <0·909 for lowest tertile, >0·909 to <1·37 for the middle tertile, and >1·37 for highest tertile. OR=odds ratio for occurrence of coronary heart disease event in the middle or lowest tertile compared with in the highest tertile.

Table 3: **Occurrence of coronary heart disease events in different tertiles of mean telomere length**

	Highest tertile (n=236)	Middle tertile (n=247)	Lowest tertile (n=253)	p
LDL cholesterol (mmol/L)	–1·24 (0·76)	–1·33 (0·83)	–1·33 (0·72)	0·495
HDL cholesterol (mmol/L)	0·047 (0·173)	–0·068 (0·168)	–0·084 (0·185)	0·158
Trigycerides (mmol/L)	–10·7 (118·0)	–6·5 (74·3)	–21·2 (50·0)	0·267
C-reactive protein (mmol/L)*	–0·199 (0·885)	–0·065 (0·836)	–0·086 (0·733)	0·339
Fibrinogen (mmol/L)	0·031 (0·981)	0·060 (0·788)	0·150 (0·858)	0·450
Plasma viscosity (mPa)	–0·013 (0·074)	–0·018 (0·080)	–0·014 (0·075)	0·841

Data are mean (SD). T/S ratios for tertile cutoffs were <0·909 for lowest tertile, >0·909 to <1·37 for the middle tertile, and >1·37 for highest tertile. *Log CRP.

Table 4: **Changes in plasma lipid levels and other markers from recruitment to end of year 1 with pravastatin treatment in individuals in different tertiles of mean telomere length**

p=0·0022 in the middle tertile; 0·55, 0·38–0·79, p=0·0015 in the lowest tertile). In multivariate analysis, accounting for all factors in table 1, the risk associated with shorter telomeres in the placebo-treated group remained significant (1·91, 1·26–2·89, p=0·0022 in the middle tertile; 2·12, 1·41–3·18, p=0·0003 in the lowest tertile; data not shown).

To see if the attenuation in risk with pravastatin treatment in individuals with shorter telomeres could be explained by differences in the effect of pravastatin on relevant intermediary markers, we compared changes in these markers from baseline to end of year 1 by telomere tertiles. There was no difference in effect of pravastatin on changes in any of the plasma lipid levels, or on CRP or fibrinogen levels in individuals with different telomere lengths (table 4).

Discussion

By use of a nested case-control approach from observations made in a prospective randomised primary prevention trial of a statin, we have shown that individuals with shorter leucocyte telomere length at the time of recruitment had a significantly higher risk of developing subsequent coronary heart disease. Interestingly, this increased risk with shorter baseline telomeres was attenuated in individuals receiving treatment with a statin. The risk of coronary heart disease associated with shorter telomeres is at least comparable to, if not greater than, more conventional risk factors (table 1 and table 3).

Our findings are consistent with previous cross-sectional studies that showed an association between shorter telomere length and coronary artery disease and premature myocardial infarction,[5,6] as well as a study that showed an association between shorter leucocyte telomeres and subsequent cardiovascular mortality in people over the age of 65 years,[7] although such an association might breakdown in a very elderly population.[19] On average, leucocyte telomere length at recruitment in individuals who developed coronary heart disease was comparable to control individuals chronologically 6 years older. One should note that such a difference was identified despite the narrow age range—45–64 years—of individuals recruited into WOSCOPS. Our findings lend support to the hypothesis that premature biological ageing might contribute to the risk of coronary heart disease.

We used a novel PCR assay, capable of high throughput, to estimate mean telomere length.[15] We confirmed that the assay is reproducible (webappendix 2) and capable of reliable identification of differences in mean telomere length by showing that it can track the attrition of mean telomere length with increasing passage number of cells in culture (webappendix 1), something that has previously been documented in several studies by Southern blotting.[4,9,11,13] The assay has been reported to show a strong correlation (r about 0·85) with mean telomere length measured by the Southern blot approach.[15,20] In our study, the correlation was slightly weaker (r=0·65; webappendix 3). This finding could be explained by the small sample size. However, one should note that, unlike the PCR-based assay, the Southern blot assay, which is done on DNA digested with restriction enzymes, also measures a variable amount of non-telomeric DNA.[15] Hence the correlation is not expected to be perfect. Finally, the observation that there was a detectable decrease in T/S ratio with increasing age in the individuals studied here is also consistent with findings from studies that used the Southern blot assay.[5,6,12] Taken together, these observations suggest that the PCR-based approach used here provides a reliable assessment of mean telomere length.

Our findings indicate that the association of shorter telomeres with coronary heart disease is not a consequence of the disease. However, whether telomere length is simply another biomarker or whether the association has a functional basis remains to be determined. The fact that cellular senescence is a major feature of atherosclerotic plaques,[2,3] and the finding that interference with telomere function in coronary endothelial cells in vitro causes expression of molecules implicated in atherogenesis,[4] suggest that shorter telomeres could conceivably contribute directly to the pathophysiology of atherosclerosis. Unlike our previous study in younger individuals with myocardial infarction (aged <50 years),[6] we did not observe a progressive increase in risk with shorter telomeres in the individuals in WOSCOPS; rather, we saw much the same risk in individuals in the middle and lowest tertile of telomere length. Analysis of the data by quintiles showed much the same increased risk in the lowest three quintiles. This finding suggests that, especially with increasing age, there could be a threshold beyond which further shortening of telomeres does not confer additional risk, consistent with the observed lack of association of telomere length with risk of coronary heart disease or mortality in very elderly individuals (aged >85 years).[19] Alternately, younger and older patients with coronary heart disease differ in other ways with respect to their risk profiles—especially their genetic susceptibility—and this could also explain the difference between the studies.

Shorter leucocyte telomeres in people prone to coronary heart disease could indicate the cumulative effect of other cardiovascular risk factors on telomere length. In particular, coronary heart disease is now thought to be a chronic inflammatory process[21] and the shorter telomere length in individuals prone to coronary heart disease could simply indicate a greater white blood cell turnover. Increased oxidative stress also contributes to atherosclerosis,[22] and increased oxidant stress has been shown to increase rates of telomere attrition in vitro.[23] In cross-sectional studies, smoking, body-mass index, and type 1 diabetes mellitus have also been reported to be associated with shorter leucocyte telomere length.[24,25] Recently, increased life stress, a factor known to increase the risk of coronary heart disease, has been shown to be associated

with shorter telomeres, possibly as a consequence of increased oxidative stress.[26] We did not observe any striking association of telomere length with several demographic characteristics or cardiovascular risk factors—including markers of systemic inflammation—in the individuals analysed here, and these factors did not confound or explain the association between shorter telomeres and increased risk of coronary heart disease. However, even if some of these markers provided a reliable assessment of inflammation, single point measurements of these variables are unlikely to accurately illustrate the cumulative burden of inflammation that is likely to affect telomere length. Thus, the absence of an association of these markers with mean telomere length does not exclude the possibility that increased chronic inflammation or oxidative stress is an important factor that contributes to the difference in telomere length between cases and controls.

An alternate, and not mutually exclusive, possibility is that the association of shorter telomeres with increased risk of coronary heart disease has a genetic basis. Several studies have shown that a substantial proportion of the marked inter-individual variation in mean telomere length is genetically determined.[27–29] This observation raises the possibility that those individuals who have inherited and are born with shorter telomeres might be more prone to coronary heart disease. Any genetic susceptibility could be exacerbated or retarded by post-natal effects on telomere length. If true, this observation could not only explain—at least in part—the genetic basis of coronary heart disease, but also its variable age of onset.

The finding of an interaction between mean telomere length and statin treament on the risk of coronary heart disease is intriguing. The observation that treatment with a statin is associated with a reduction in the number of clinical events, but only in those individuals with increased risk based on their telomere length, suggests that telomere length has a primary role in increasing the risk of coronary heart disease. The mechanism of such an effect seems to be independent of the effect of statins on lipid levels or markers of inflammation, since we noted that there was no difference in changes in plasma concentrations of LDL cholesterol, HDL cholesterol, triglycerides, CRP, or fibrinogen levels with statin treatment in people with different telomere lengths. A potential mechanism is suggested by the recent observation that treatment of endothelial progenitor cells with a statin results in a threefold increase in the expression of the telomere capping protein TRF2.[30] Since the loss of TRF2 can induce telomere dysfunction, this finding raises the possibility that the observed attenuation of risk in individuals with shorter telomeres by statin treatment is at least in part mediated through increased TRF2 expression in endothelial and other relevant cells and stabilisation of telomeric structure. Statins have also been shown to enhance mobilisation of bone-marrow derived endothelial progenitor cells, which have recently been implicated in vascular repair.[31,32] The association between telomere shortening and endothelial senescence in vitro suggests that the need for such repair might be greater in those individuals with shorter telomeres; enhanced mobilisation of endothelial progenitor cells by statins could also explain the attenuation of risk in such individuals. Thus either telomere-dependent or telomere-independent processes could explain the interaction between telomere length and benefit from statin treatment on the risk of coronary heart disease.

Our study has important limitations. Although leucocytes provide an easily accessible source for DNA analysis, the relevance of measurement of telomere length in these circulating cells to processes that occur in the coronary vasculature remains to be established. The limited data that are available suggest that there are correlations between telomere length in different tissues of an individual that indicate the genetic determination of telomere length.[33] There is also evidence that, against this background, telomere length attrition is accelerated in vascular sites prone to atherogenesis.[34] However, further studies are needed to determine whether the prospective association of telomere length with the risk of coronary heart disease also applies to telomere length in other cell types and especially vascular tissue.

In summary, we have shown that leucocyte telomere length is associated with future coronary heart disease events in middle-aged, high-risk men, and that it could identify those individuals who would benefit most from statin treatment. These findings could have important implications for our understanding of the pathophysiology of coronary heart disease and, in particular, the role of biological ageing.

Contributors

S W Brouilette and J S Moore undertook all the laboratory analysis. A D McMahon and J R Thompson did the statistical analysis and contributed to interpretation of the data. I Ford, J Shepherd, and C J Packard helped with the conduct of the study and interpretation of the findings. N J Samani conceived and designed the study and supervised the conduct of the study. N J Samani and S W Brouilette drafted the manuscript, which was contributed to by all authors. All authors saw and approved the final version of the manuscript.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This work was supported by grants from the Sir Jules Thorn Charitable Trust and the British Heart Foundation. NJS holds a Personal Chair from the British Heart Foundation. We thank S M Cobbe, P W Macfarlane, and C G Isles, who are active members of the West of Scotland Coronary Prevention Study Group.

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