

White Cell Telomere Length and Risk of Premature Myocardial Infarction

Scott Brouillette, Ravi K. Singh, John R. Thompson, Alison H. Goodall, Nilesh J. Samani

Objective—Biological age may be distinct from chronological age and contribute to the pathogenesis of age-related diseases. Mean telomeres lengths provide an assessment of biological age with shorter telomeres, indicating increased biological age. We investigated whether subjects with premature myocardial infarction (MI) had shorter leukocyte telomeres.

Methods and Results—Mean terminal restriction fragment (TRF) length, a measure of average telomere size, was compared in leukocyte DNA of 203 cases with a premature MI (<50 years) and 180 controls. Age- and sex-adjusted mean TRF length of cases was significantly shorter than that of controls (difference 299.7 ± 69.3 base pairs, $P < 0.0001$) and on average equivalent to controls 11.3 years older. The difference in mean TRF length between cases and controls was not accounted for by other coronary risk factors. Compared with subjects in the highest quartile for telomere length, the risk of myocardial infarction was increased between 2.8- and 3.2-fold ($P < 0.0001$) in subjects with shorter than average telomeres.

Conclusions—The findings support the concept that biological age may play a role in the etiology of coronary heart disease and have potentially important implications for our understanding of its genetic etiology, pathogenesis, and variable age of onset. (*Arterioscler Thromb Vasc Biol.* 2003;23:842-846.)

Key Words: myocardial infarction ■ telomeres ■ coronary disease ■ genetics ■ aging

Although epidemiological studies have identified several important risk factors predisposing to coronary heart disease (CHD) and its clinical sequelae, a significant proportion of the risk still remains unexplained, and novel mechanisms warrant consideration. CHD is an age-related disease, and therefore premature biological as distinct from chronological aging may contribute to its risk.

Telomeres are the extreme ends of chromosomal DNA, made up of a large number of tandem repeats of the sequence TTAGGG. Although the functions of telomeres are not fully understood, they are involved in maintaining cellular stability.^{1,2} Because DNA polymerase cannot fully complete the replication of the 3' end of linear DNA, telomeres progressively shorten with repeated cell division.³ In 1973, Olovnikov⁴ was the first to suggest that this is a potential mechanism for a biological clock determining cellular behavior, and this concept has since been supported experimentally.^{5,6} In many cell types, senescence and subsequent cell death often occurs when the mean telomere length reaches a critical value.⁷ Therefore, mean telomere length provides a marker for biological age, at least at the cellular level, with shorter telomeres indicating increased biological age.

In a previous study on a small cohort of subjects,⁸ we observed that the mean telomere restriction fragment (TRF)

length, a measure of mean telomere size, was significantly shorter in circulating white cells of patients with severe (triple-vessel) CHD compared with matched subjects with normal coronary angiograms. The finding has raised several important questions, not least whether the association is genuine and, if so, whether it is primary or secondary to an effect of other risk factors for CHD on white cell telomere attrition rate. We hypothesized that, if it is primary, an important clinical consequence could be a predisposition to premature myocardial infarction. In this study, we therefore examined whether there is an association between white cell TRF length and premature myocardial infarction in a large cohort of patients and age- and sex-matched controls and investigated whether any association can be explained by other more-established risk factors for CHD.

Methods

Subjects

The cases comprised 203 subjects recruited retrospectively from the registries of 3 coronary care units in Leicester. All had suffered a myocardial infarction according to WHO criteria before the age of 50 years. Diagnosis was verified by inspection of hospital records. At the time of participation, all case subjects were at least 3 months from their acute event and in a clinically stable condition. The control cohort comprised 180 subjects with no personal or family

Received December 3, 2002; revision accepted February 24, 2003.

From the Division of Cardiology, Department of Medicine (S.B., R.K.S., N.J.S.), Department of Epidemiology and Public Health (J.R.T.), and Department of Clinical Biochemistry (A.H.G.), University of Leicester, United Kingdom.

Correspondence to Dr Nilesh J. Samani, Division of Cardiology, University of Leicester, Clinical Sciences Wing, Glenfield Hospital, Groby Rd, Leicester, LE3 9QP, UK. E-mail njs@le.ac.uk

© 2003 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at <http://www.atvbaha.org>

DOI: 10.1161/01.ATV.0000067426.96344.32

history of premature coronary heart disease, matched for age, sex, and current smoking status with the cases. Control subjects were recruited from 3 primary care practices located within the same geographical area. All subjects were white of Northern European origin. The study was approved by the Leicestershire Health Authority Ethics Committee, and all subjects provided written informed consent.

Measurements

A standard questionnaire was filled in on all subjects regarding personal and family history and drug therapy. Subjects were categorized as suffering from hypertension and diabetes on the basis of reported history. Smoking status was defined as current smoker, ex-smoker and nonsmoker. Serum cholesterol was measured using standard reagents (7D62-01) on the AEROSET system (Abbott Laboratories). White cell count was measured in EDTA blood using a Beckman Coulter A^CT diff counter (Beckman Coulter Inc). C-reactive protein (CRP) was measured using the Olympus high-sensitivity CRP Immunoassay (catalogue No. OSR6185) on an Olympus AU 400 Analyser (Olympus America Inc). Fibrinogen was measured by the Clauss method on a Sysmex CA-1000 analyser (Sysmex UK). Homocysteine was measured by high-power liquid chromatography with fluorescence detection using a commercially available kit (Cat No. 4500, Chromsystems Ltd).

Terminal Restriction Fragment Analysis

This was performed as previously described.^{3,5-8} and blinded to the clinical data. Briefly, leukocyte DNA was extracted from peripheral blood samples using PureGene DNA Extraction Kits (Gentra Systems) and the quality assessed by agarose gel electrophoresis. Aliquots of DNA (8 µg) were digested overnight at 37°C with 15U *RsaI* and *HinfI* (Invitrogen) and quantitated by fluorometry, and 2 µg of each sample was then resolved by electrophoresis on a 0.5% agarose gel (50V, 18 hours). Size standards were run on either side of the gel (500 ng KB DNA, Invitrogen, and 700 ng Hyperladders I and VI, Bionline). After transfer to Hybond-N membrane (AmershamPharmacia Biotech) using a BioRad 583 Gel Dryer (room temperature for 30 minutes followed by 65°C for 30 minutes), DNAs were hybridized at 42°C overnight to a 3'-end-labeled ³²P-(AATCCC)₃ oligonucleotide telomere probe and random primed labeled KB DNA ladder (30 ng) and Hyperladders I and VI (45 ng). After washes in 3×standard saline citrate/0.1% sodium dodecyl sulfate at room temperature, telomere smears were visualized by exposure to autoradiographic film (Kodak Biomax-MR, Eastman Kodak) and digitized using a Phosphor-imager (Molecular Dynamics). IMAGEQUANT software (Molecular Dynamics) was used to analyze telomere smears by drawing a grid object (1 column×30 rows) over each lane, from 24 to 2.5 kb. The mean size of the terminal restriction fragment (TRF) was estimated using the formula $TRF = [(OD_i)/(OD_i/MW_i)]$, where OD_i is the optical density at a given position on the gel and MW_i is the molecular weight at that position.^{3,8} A single control sample was run on each gel to adjust for inter-gel variability. The interassay variance in estimate of mean TRF length was calculated from repeat analysis of 50 samples selected at random and was 3.3±2.7%.

Statistical Analysis

Characteristics of cases and controls were compared using unpaired *t* test for continuous variables and χ^2 test for categorical variables. The effects of age, sex, case/control status, and other individual risk factors on mean TRF length were assessed using regression models, with adjustments as described in the text. Mean TRF length as a risk factor for MI was assessed using logistic regression. All analysis was carried out in Stata Statistical Software (Release 7.0; Stata Corporation, 2001).

Results

The demographic characteristics of the subjects are shown in Table 1. Cases and controls were well matched for age and

TABLE 1. Demographics of Cases and Controls

	Cases (n=203)	Controls (n=180)	P
Age, y*	46.8 (6.2)	47.2 (5.9)	0.483
M:F, %*	85:15	86:14	0.934
Age at time of MI, y	42.3 (5.7)	NA	...
Current/ex-/nonsmokers, %*	21:61:18	17:33:50	<0.0001
Hypertension, %	28.1	8.3	<0.0001
Diabetes, %	10.8	1.1	<0.0001
Positive family history†	59.6	NA	...
White cell count, ×10 ⁶ /mL	6.85 (0.15)	5.43 (0.10)	<0.0001
Plasma cholesterol, mmol/L	4.92 (1.28)	5.22 (1.02)	0.015
Plasma fibrinogen, mg/dL	3.14 (0.74)	2.80 (0.57)	<0.0001
Plasma CRP, mmol/L‡	2.55 (5.68)	1.92 (1.99)	0.787
Plasma homocysteine, mmol/L	12.25 (0.29)	11.88 (0.31)	0.377

Data are shown as percentages for categorical variables and means (SD) for continuous variables.

NA indicates not applicable.

*Characteristics used for matching (note that for smoking, this was for current smoking, and the significant *P* value reflects the difference in the number of previous and nonsmokers between the groups).

†History of CHD in first degree relative younger 65 years of age.

‡The CRP distributions were skewed, and the *P* value is from log-transformed data.

sex. The mean age at event of the cases was 42.3±5.7 years. As expected, cases had a higher prevalence of diabetes and hypertension, and more were current or ex-smokers. Plasma cholesterol level at the time of study was significantly lower in cases than controls, but this likely reflects the fact that a high proportion of the cases (76.8%) were taking lipid-lowering medication. White cell count and plasma fibrinogen level were significantly higher in cases compared with controls, but there was no significant difference in plasma C-reactive protein or homocysteine levels. Almost 60% of cases reported a history of CHD before the age of 65 years in a first-degree relative.

The mean white cell TRF length as a function of age is shown in Figure 1 for both cases and controls. Despite the relatively narrow age range, highly significant (*P*<0.0001) decreases in mean white cell TRF length with increasing age were observed in both groups. The decrease per year was 28.3±7.0 base pairs (bp) in cases and 24.8±9.3 bp in controls. This difference was not significant (*P*=0.757).

Age-adjusted mean TRF lengths for cases and controls were 6.77±0.17 kb for male cases, 6.62±0.18 kb for female cases, 7.05±0.16 kb for control males, and 7.02±0.11 kb for control females. There was no independent effect of sex on mean TRF length (*P*=0.327). However, taking age and sex into account, the mean TRF length of cases was highly significantly lower compared with controls (difference 299.7±69.3 bp, *P*<0.0001).

An analysis of the impact of other cardiovascular risk factors on mean TRF length is summarized in Table 2. A history of hypertension was associated with mean white cell TRF length that was almost 100-bp shorter, but this did not reach statistical significance. No independent effects were found for either a history of diabetes or smoking (assessed

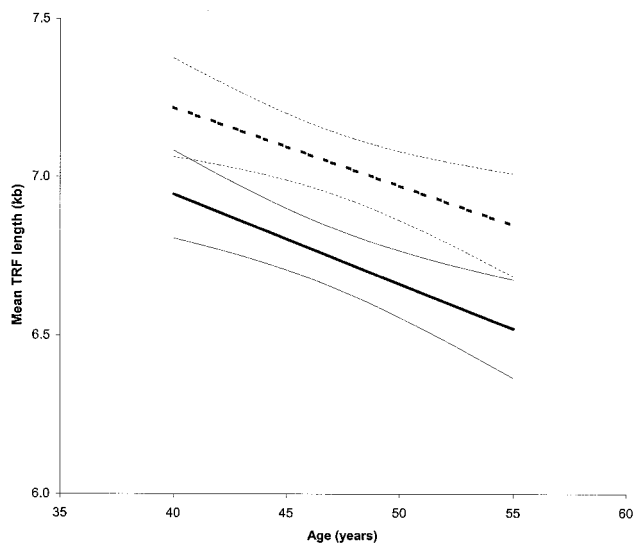


Figure 1. Age-related decrease in mean TRF length in cases and controls. Declines in mean white cell TRF length with age in cases (solid lines) and controls (hatched lines) are shown separately. The bold lines represent the average and the lighter lines the 95% confidence intervals for each group. The regression lines extend beyond 50 years because, although all the MI cases had their incident event before the age of 50 years, some (and the respective controls) were recruited up to age 55 years. For clarity, only the regression lines are shown, although a graph showing all the subjects is available from the corresponding author.

either in terms of smoking status or pack years). The relationship with cholesterol level was assessed in the control group only because of the possible confounding by statin therapy in the cases. There was no significant relationship between plasma cholesterol level and telomere length. There

TABLE 2. Effects of CHD Risk Factors on Mean TRF Length

Variable	Effect on Mean TRF Length, bp	P
History of hypertension (n=72)	-99.2 (92.7)	0.285
History of diabetes (n=24)	+1.3 (146.8)	0.993
Smoking		
Current (n=72)	-63.8 (103.8)	0.539
Ex-smoker (n=185)	+8.2 (84.6)	0.923
Per pack year	-2.3 (2.0)	0.264
Per mmol/L higher cholesterol*	-67.5 (55.9)	0.229
Per 1×10^6 higher white cell count	-17.0 (20.3)	0.404
Per mmol/L higher fibrinogen	-21.1 (54.5)	0.699
Per mmol/L higher CRP	-4.4 (8.0)	0.583
Per mmol/L higher homocysteine	+12.2 (8.5)	0.149
Positive family history (CHD in 1st degree relative <65 years old)†	-114.8 (88.4)	0.195
Positive family history (MI in first degree relative <50 years old) †	-152.5 (94.3)	0.107

Mean effect (SE) after adjustment for age, sex, and case/control status are shown. A negative value indicates that the variable was associated with shorter mean TRF length.

*Only in controls (see Results).

†Only in cases, see legend to Table 1 for definition.

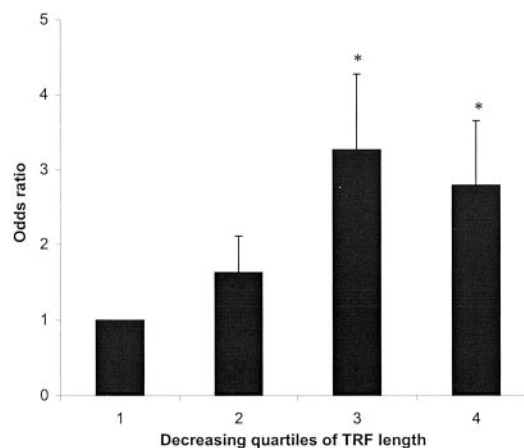


Figure 2. Risk of myocardial infarction with different telomere lengths. The figure shows the odds ratio (+SE) of risk of MI in different quartiles of mean TRF length compared with the highest quartile (1). * $P \leq 0.001$ compared with quartile 1.

was also no significant relationship between mean TRF length and either white cell count or plasma levels of C-reactive protein, fibrinogen, or homocysteine. Interestingly, in case subjects, there was a trend toward shorter mean TRF length in those with a positive family history of premature CHD (especially in those with a first-degree relative with an MI before age 50 years), although this did not reach significance (Table 2). Input of all of these factors into a logistic regression analysis did not affect the relationship between case status and shorter TRF length (difference 315.7 ± 84.9 bp, $P = 0.0002$).

The odds ratios of MI associated with different mean TRF lengths are shown in Figure 2. Compared with subjects in the highest quartile for TRF, the odds ratios were 1.63 (95% CI, 0.91 to 2.92; $P = 0.102$), 3.27 (95% CI, 1.79 to 5.97; $P < 0.0001$), and 2.79 (95% CI, 1.53 to 5.11; $P = 0.001$), respectively, in subjects in the second, third, and lowest quartile of mean TRF length.

Discussion

In this study, we report a highly significant and strong association between mean leukocyte TRF length and premature myocardial infarction in a well-characterized large case-control study. Having shorter than average mean leukocyte TRF length increased the risk of MI by approximately 3-fold. The magnitude of the relationship is additionally underscored by the fact that, if the average decline per year in TRF length is taken as 26.4 bp per year (mean of cases and controls), then the difference in mean leukocyte TRF length between cases and controls (299.7 bp) represents a biological age gap of more than 11 years. This is despite the relatively narrow chronological age range of the subjects.

The findings substantiate and greatly extend the results of a previous small study (10 cases and 20 controls), where we found shorter leukocyte telomeres in subjects with advanced coronary atherosclerosis compared with age-matched subjects with normal coronary angiograms.⁸ Here we have studied the disease process at a much earlier age, where any differences are more likely to reflect primary mechanisms.

Importantly, we have also examined whether other recognized risk factors for CHD explain the observed association. Shorter white cell telomeres have been associated with insulin-dependent (but not type 2) diabetes mellitus⁹ and also pulse pressure and pulse wave velocity,^{10,11} indices of large artery stiffness related to hypertension. Although there was a trend toward shorter telomeres in subjects who were hypertensive (Table 2), we found no significant effect of a history of either hypertension or diabetes mellitus (mainly type 2 in our subjects) on mean leukocyte TRF length, and certainly neither of these CHD risk factors nor the excess history of smoking in cases explain the observed association.

There are compelling data implicating low-grade inflammation¹² and increased oxidative stress¹³ in the pathogenesis of coronary atherosclerosis. White cell count as well as plasma levels of inflammatory markers such as CRP and fibrinogen have been shown to be strong predictors of future events.^{14,15} *In vitro*, telomere attrition has been shown to be accelerated by oxidative stress,¹⁶ and homocysteine, a recognized risk factor for atherosclerosis,¹⁷ has been found to increase the amount of telomere length lost per population, doubling in endothelial cells through a redox-dependent pathway.¹⁸ To explore the possibility that the shorter telomeres in subjects with premature MI represent either increased white cell turnover or increased telomere loss per mitosis related to either chronic inflammation or oxidative stress, we assessed the impact of several relevant parameters. Consistent with previous reports,¹⁵ both fibrinogen level and white cell count were significantly higher in cases, and there was a nonsignificant trend toward higher CRP level. However, neither these factors nor the plasma homocysteine level had a significant independent effect on mean TRF length, and in a logistic regression analysis, none of these factors affected the association of shorter mean TRF length with risk of MI. Indeed, adjustment for these variables and the demographic risk factors slightly increased the difference in mean TRF length between cases and controls.

The early separation of the mean TRF lengths of cases and controls, coupled with the similar rate of attrition with age (Figure 1), point toward a possible primary genetic basis for the difference. This is supported by results from both twin¹⁹ and family studies,¹⁰ which have found that, to a significant extent (75% to 80%), interindividual differences in mean TRF length in blood are genetically determined. If this explanation is correct, then there are several provocative and potentially important implications of our findings. First, individuals born with relatively shorter telomeres may be at higher risk of premature CHD. A genetic tendency to having shorter telomeres could also, at least partly, explain the well-established increased familial basis of premature CHD.^{20,21} In this regard, the finding that those case subjects with a strong family history showed a trend toward shorter telomeres (Table 2) is noteworthy. An important corollary of this possibility is that to date investigation of the molecular basis of the genetic contribution to risk of premature CHD has exclusively focused on the role of individual genes²¹ rather than a more global property of the genetic material. This may need to be reappraised. Finally, interindividual variation in telomere length could explain some of the

variability in age of onset of CHD that exists even when conventional risk factors are taken into account. These possibilities are necessarily speculative at this stage but potentially worthy of investigation if additional studies confirm the association. For example, it would be interesting to see if telomere lengths differ in unaffected first-degree relatives of those with and without CHD, especially in relatives who do not manifest other major risk factors for cardiovascular disease.

There is both *in vivo* and *in vitro* evidence suggesting that cellular senescence plays a role in the pathogenesis of atherosclerosis. Morphological changes consistent with senescence have been demonstrated in endothelium overlying atherosclerotic plaques.^{22–24} More importantly, such cells have been shown to express increased levels of plasminogen activator inhibitor type 1¹⁸ and intracellular adhesion molecule-1²⁵ and decreased levels of nitric oxide,²⁴ all alterations that are implicated in atherogenesis.²⁶ Recently, Minamino et al²⁴ have shown that some of these functional changes are directly related to telomere biology in human vascular endothelial cells. When telomere dysfunction was induced by inhibition of the telomeric protein TRF2, phenotypic changes characteristic of senescence occurred and the cells exhibited increased intracellular adhesion molecule-1 expression and decreased endothelial nitric oxide synthase activity.²² In contrast, introduction of telomerase catalytic component (which protects telomeres from shortening) significantly extended the life span and inhibited the functional alterations associated with senescence.²⁴ Therefore, there are plausible mechanisms linking shorter telomeres to the development of atherosclerosis, although additional studies are clearly required.

Several limitations of our study need to be considered. In particular, a case-control study cannot exclude the possibility that an unmeasured factor accounts for an observed relationship. Specifically, the cases in our study represent the survivors of a MI. Up to 30% of subjects who suffer a MI do not survive the acute event. Although unlikely, we cannot therefore exclude the possibility that possessing longer telomeres adversely affects the immediate prognosis after MI and that this explains the observed association. Replication of the data ideally in prospective studies is therefore essential. Also, we measured mean TRF length in the whole white cell population, and it would be interesting to know whether there are differences between the different white cell types. Additional studies are also necessary to show that subjects with premature MI have shorter telomeres in the cells of their coronary vasculature. However, it has been shown that telomere loss as a function of donor age is greater in intimal DNA from iliac arteries, a site exposed to greater hemodynamic stress and prone to atherosclerosis, compared with that of internal thoracic arteries, which does not demonstrate atherosclerosis.²⁷ Therefore, it is likely that an increased rate of cell turnover in vascular trees subject to disturbed flow, including the coronaries, will, if anything, accelerate telomere loss and exacerbate any inherited abnormality. Lastly, measurement of mean TRF length is a relatively crude measure. Telomere lengths may vary not only among different cell types but also among chromosomes.²⁸ Telomere attrition

might therefore affect gene function of a subset of chromosomes long before replicative capacity is impaired. Such a situation may have particular relevance to chronic processes such as atherosclerosis. Therefore, more information is also needed about both the process of telomere shortening and its impact on cellular function.

In summary, we report a novel and powerful association of shorter leukocyte telomeres with risk of premature MI that is independent of other risk factors or markers of inflammation. If the finding is replicated in additional studies, it could represent a primary mechanism, which could have important implications for our understanding of the genetic etiology and pathogenesis of coronary atherosclerosis and at least provide a partial explanation for the variable age of occurrence of its clinical sequelae.

Acknowledgments

S. Brouillette is supported by a Medical Research Council PhD studentship; R.V. Singh was supported during the course of this work by a British Cardiac Society Fellowship.

References

- Blackburn EH. Structure and function of telomeres. *Nature*. 1991;350:569–573.
- Zakian VA. Telomeres: beginning to understand the end. *Science*. 1995;270:1601–1607.
- Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature*. 1990;345:458–460.
- Olovnikov AM. A theory of marginotomy. *J Theor Biol*. 1973;41:181–190.
- Vaziri H, Dragowska W, Allsopp RC, Thomas TE, Harley CB, Lansdorp PM. Telomere length predicts replicative capacity of human fibroblasts. *Proc Natl Acad Sci U S A*. 1992;89:10114–10118.
- Vaziri H, Dragowska W, Allsopp R, Thomas T, Harley CB, Lansdorp PM. Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proc Natl Acad Sci U S A*. 1994;91:9857–9860.
- Allsopp RC, Harley CB. Evidence for a critical telomere length in senescent human fibroblasts. *Exp Cell Res*. 1995;219:130–136.
- Samani NJ, Boulby R, Butler RB, Thompson JR, Goodall AH. Telomere shortening in atherosclerosis. *Lancet*. 2001;358:472–473.
- Jeanclous E, Krolewski A, Skurnick J, Kimura M, Aviv H, Warram JH, Aviv A. Shortened telomere length in white blood cells of patients with IDDM. *Diabetes*. 1998;47:482–486.
- Jeanclous E, Schork N, Kyvik KO, Kimura M, Skurnick JH, Aviv A. Telomere length inversely correlates with pulse pressure and is highly familial. *Hypertension*. 2000;36:195–200.
- Benetos A, Okuda K, Lajemi M, Kimura M, Thomas F, Skurnick J, Labat C, Bean K, Aviv A. Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension*. 2001;37:381–385.
- Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med*. 1999;340:115–126.
- Harrison D. Oxidative stress and coronary artery disease. *Can J Cardiol*. 1998;14(suppl D):30D–32D.
- Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and risks of cardiovascular disease in apparently healthy men. *N Engl J Med*. 1997;336:973–979.
- Packard CJ, O'Reilly DS, Caslake MJ, McMahon AD, Ford I, Cooney J, Macphee CH, Suckling KE, Krishna M, Wilkinson FE, Rumley A, Lowe GD. Lipoprotein-associated phospholipase A2, as an independent predictor of coronary heart disease. *N Engl J Med*. 2000;343:1148–1155.
- von Zglinicki T. Role of oxidative stress in telomere regulation and replicative senescence. *Ann NY Acad Sci*. 2000;908:99–110.
- Eikelboom JW, Lonn E, Genest J Jr, Hankey G, Yusuf S. Homocysteine and cardiovascular disease: a critical review of the epidemiologic evidence. *Ann Intern Med*. 1999;131:363–375.
- Xu D, Neville R, Finkel T. Homocysteine accelerates endothelial cell senescence. *FEBS Lett*. 2000;470:20–24.
- Slagboom PE, Droog S, Boomsma DI. Genetic determination of telomere size in humans: a twin study of three age groups. *Am J Hum Genet*. 1994;55:876–882.
- Marenberg ME, Risch N, Berkman LF, Floderus B, de Faire U. Genetic susceptibility to death from coronary heart disease in a study of twins. *N Engl J Med*. 1994;330:1041–1046.
- Samani NJ, Singh RK. What is known about the genetics of acute coronary syndromes. In: de Bono DP, Sobel E, eds. Current challenges in acute coronary syndromes. Oxford: Blackwell Science; 2001:81–98.
- Davies MJ, Woolf N, Rowles PM, Pepper J. Morphology of the endothelium over atherosclerotic plaques in human coronary arteries. *Br Heart J*. 1988;60:459–464.
- Burrig KF. The endothelium of advanced arteriosclerotic plaques in humans. *Arterioscler Thromb*. 1991;11:1678–1689.
- Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I. Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. *Circulation*. 2002;105:1541–1544.
- Comi P, Chiaromonte R, Maier JAM. Senescence-dependent regulation of type I plasminogen activator inhibitor in human vascular endothelial cells. *Exp Cell Res*. 1995;219:304–308.
- Lusis AJ. Atherosclerosis. *Nature* 2000; 407:233–241.
- Chang E, Harley CB. Telomere length and replicative aging in human vascular tissues. *Proc Natl Acad Sci U S A*. 1995;92:11190–11194.
- Lansdorp PPM, Verwoerd NP, van de Rijke FM, Dragowska V, Little MT, Dirks RW, Raap AK, Tanke HJ. Heterogeneity in telomere length of human chromosomes. *Hum Mol Genet*. 1996;5:685–691.