Telomere shortening in atherosclerosis

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Eukaryotic chromosomes end with telomeres, which shorten with cellular ageing. We investigated whether atherosclerosis is associated with systemic evidence of accelerated cellular ageing. We compared mean length of terminal restriction fragments (TRF), a measure of average telomere size, in leucocyte DNA of ten patients with severe coronary artery disease (CAD) with that of 20 controls without CAD. Adjusting for age and sex, cases had mean TRF lengths of 303 (SD 90) base pairs shorter than those of controls (p=0·002)—ie, equivalent in size to individuals with no CAD who are 8·6 years older. Although this is a pilot study, the findings could be relevant to the pathogenesis of atherosclerosis.

Lancet 2001 **358:** 472–73

The development of atherosclerotic plaques is characterised by increased local cell turnover in response to injury.[1] Because most somatic cells can only undergo a finite number of divisions before reaching senescence, this leads to biological ageing of the cells and a decline in their replicative potential. In turn, the cellular dysfunction that accompanies senescence of vascular cells could contribute to the development of atherosclerosis. Although atherosclerotic disease is focal, there is increasing evidence that it is accompanied by more generalised perturbations, and in particular systemic inflammation.[1] Thus, presence of atherosclerosis could be marked by increased cell turnover and increased biological age in other cell types. Eukaryotic chromosomes end with telomeres, which comprise tandem repeats of the sequence TTAGGG extending over several kilobases (kb), and are involved in the stabilisation of chromosomal ends.[2] At mitosis, like other chromosomal regions, telomeres are replicated by DNA polymerase. However, because DNA polymerase cannot fully complete the replication of the 3′ end of linear DNA molecules, telomeres progressively shorten with repeated cell division, and senescence occurs when the mean telomere length reaches a critical value.[2,3] Thus, telomere length can be viewed as a biomarker of somatic cell ageing. We examined whether patients with advanced coronary atherosclerosis (CAD) show systemic evidence of telomeric shortening compared with individuals without CAD.

Leucocyte DNA was extracted from peripheral blood samples of ten patients (age 42–72 years, nine men and one woman) with angiographically detected severe (>75% stenosis) triple-vessel CAD, and 20 control participants (age 39–72 years, ten men and ten women) with normal arteries on angiogram. Control participants underwent angiography for investigation of either valvular heart disease (n=9) or chest pain of uncertain origin (n=11). We used the standard method to measure mean length of terminal restriction fragments (TRF), a measure of mean telomere length.[3] Briefly, 2 μg DNA, digested with 15 U RsaI and HinfI, was resolved overnight at 50 V in a 0·5% agarose gel (with size markers), blotted to a nylon filter and hybridised to a radio-labelled 18-mer ($AATCCC_3$) synthetic oligonucleotide probe complementary to the telomere tandem repeat sequence. After washing, filters were exposed to a phosphor-imager to obtain an image of the telomere smear, which was analysed by Imagequant software (Molecular Dynamics, Sunnyvale, California, USA, version 3·3). The mean length of TRFs was calculated with the formula:

$$TRF=\Sigma OD_i / (\Sigma OD_i/MW_i)$$

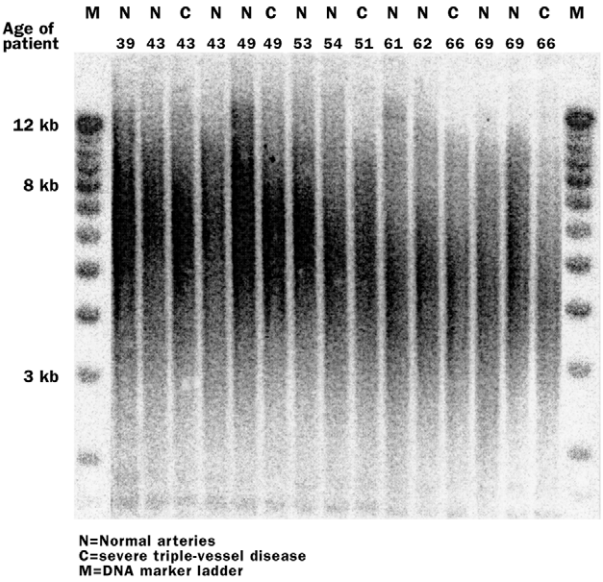

Figure 1: **Representative autoradiogram of Southern hybridisation of telomeric DNA from individuals with angiographically normal coronary arteries or with severe triple-vessel disease**

where OD_i is the optical density at a given position in the gel and MW_i is the molecular weight at that position. A control sample was run in each gel to adjust for intergel variability, and all DNAs were assayed at least twice. The interassay variation in mean TRF was 0·8% (standard deviation 1·5%).

An example of an autoradiogram showing the distribution of TRFs in leucocytes of cases and controls is shown in figure 1, and the mean TRF length for each participant, plotted by age is shown in figure 2. There was a highly significant (p<0·001) effect of age on mean TFR with an average decrease in length equivalent to 35 (standard error [SE]=4) base pairs (bp) per year of life. Adjusting for age and sex, patients with CAD had mean TRF lengths of 303 (SE=90) bp shorter than those of controls (p=0·002; figure 2). Although there was a sex imbalance and the number of participants was small, we believe no bias was introduced. There was no independent effect of sex (p=0·543) and the difference in mean TRF between cases and controls remained significant when the analysis was restricted to the males (difference 309 [SE 67] bp, p<0·001).

Our study supports previous observations of an age-related decrease in mean TRF length in circulating leucocytes of 30–40 bp/year in individuals with no CAD,[3] reflecting turnover in the bone marrow, but also shows significantly shorter telomeres in leucocytes of individuals with CAD compared with those without. On average, mean TRF length in individuals with CAD was similar to those without, but who were 8·6 years older (calculated from the mean difference in TRFs between cases and controls [303 bp] and the mean annual decrease in telomere length [35 bp]). A plausible explanation for the shorter telomeres in leucocytes in patients with CAD is that it reflects increased leucocyte turnover as a consequence of the systemic chronic inflammation that is now believed to occur in atherosclerosis.[1] Equally, this difference could represent the effect of other risk factors for atherosclerosis. Within the constraints of the limited numbers, there was no significant effect of current smoking, diabetes, hypertension, or serum cholesterol, on either the difference in mean TRF lengths between our cases and controls or the decrease in mean

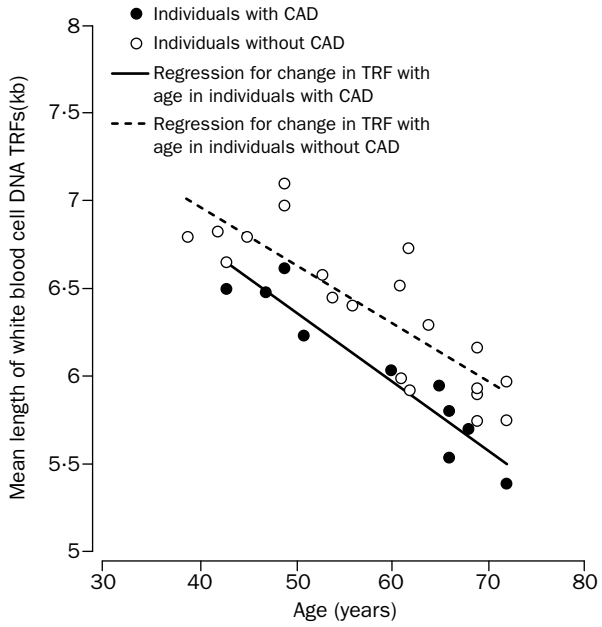

Figure 2: **Plot of mean length of leucocyte DNA terminal restriction fragments against age of individuals with severe coronary artery disease or normal coronary arteries**
TRF=terminal restriction fragments; CAD=coronary artery disease.

TRF length with age, but larger studies are required to investigate this. Other explanations also need to be considered. Specifically, decreased telomere length in those prone to develop CAD may be set at an early stage (figure 2). This raises the possibility of two other explanations. First, that the difference is genetically mediated. Slagboom and colleagues did a study of twins in which they found that 78% of interindividual differences in mean TRF length in blood is genetically determined.[4] Alternatively, the shorter leucocyte telomeres in individuals with CAD could reflect a period of increased replicative stress during intrauterine or early post-natal life. Such a period of catch up growth is envisioned in the hypothesis proposed by Barker and co-workers in which they link dysfunctional growth in early life and late-onset cardiovascular disorders, including atherosclerosis.[5] The important implication of either of these explanations is that, if true, the shorter telomeres in leucocytes (and by inference other tissues) change from simply being a marker of the atherosclerotic process and become a primary abnormality, which provides a substrate for accelerated biological ageing and cellular senescence in response to other atherosclerotic risk factors. As such, our observation could bring together several different strands concerning the aetiology and pathogenesis of CAD.

We thank Nicola Royle and Simon Frantz for advice about technical aspects of the telomere assay. Rachel Boultby received a Medical Research Council studentship and, the study was supported by the British Heart Foundation (PG/ 97196).

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The incidence of out-of-hospital ventricular fibrillation in Helsinki, Finland, from 1994 to 1999

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Early defibrillation by emergency medical services has been a success story in the treatment of ventricular fibrillation. This success has been followed by recommendations to allow public access to defibrillation equipment. We tracked the changes in incidence of ventricular fibrillation from prospectively collected data from the Helsinki Cardiac Arrest Register. We found that the incidence of out-of-hospital ventricular fibrillation of cardiac origin fell by 48% from 1994 to 1999 (p=0·0036). The primary and secondary prevention of coronary artery disease may not be the only reasons for this change and so new public-access defibrillation programmes should be delayed until our findings are confirmed.

Lancet 2001; **358:** 473–74

Ventricular fibrillation (VF) is the most common (≤80%) initial rhythm in out-of-hospital cardiac arrest. The concept of early defibrillation has been successfully adopted by emergency medical services. The hospital discharge rate after VF can reach 42% in areas where the emergency medical services provide an efficient and competent care (eg, in Göttingen, Germany).[1] Major investments are being made to set-up public-access defibrillation equipment in strategic positions—ie, in public places where the incidence of cardiac arrest is at least 0·2 per 100 000 per year or call-to-shock interval is more than 5 min.[2] However, public-access defibrillation is of no help if the initial rhythm is not one of VF or ventricular tachycardia.

Since 1994 data from out-of-hospital cardiac arrests in the area covered by the Helsinki Emergency Medical Services have been collected and analysed according to the Utstein style.[3] The emergency medical services is three tiered and fire-brigade based and serves the whole 551 000 population of Helsinki. All units are equipped with semiautomatic defibrillators to be used in case of cardiac arrest. The diagnosis of VF was based on analysing algorithms of semi-automatic defibrillators (Heartstart 2000 or 3000, Laerdal, Stavanger, Norway) confirmed afterwards by an emergency medical services physician checking the data cards. We report the change in the incidence of bystander witnessed VF of cardiac origin from 1994 to 1999 derived from Helsinki Cardiac Arrest Register (table).

There was a 48% reduction in the incidence of out-of-hospital VF over the study period (p=0·0036). The findings were not explained by more prompt reaction time by patients to preceding symptoms (eg, chest pain, dyspnoea) before the occurrence of cardiac arrest. There was no significant change in the time delay from the onset of pain to emergency call in acute myocardial infarction (the annual decrease was 0·5 min [95% CI –8·0–7·1], p=0·90) or in the occurrence of VF witnessed by a member of the emergency medical services (number for respective