Supplemental Material

Data S1.

Protocol for leukocyte telomere length analysis by the terminal restriction fragment length (TRFL analysis)

A major problem with the leukocyte telomere length (LTL) analysis is that the DNA may migrate at slightly different rates in different regions of the gel. This is due to a host of technical reasons. For this reason, we have developed techniques that resolved each digested DNA sample and the molecular weight ladder on the same lane, thereby eliminating the effect of variation in DNA migration in different lanes. Samples are digested overnight with restriction enzymes digest set, HinfI (5.2 U)/Rsa I (5.2 U) (Roche). DNA samples (3 µg each) and DNA ladders (1 kb DNA ladder plus 23.1kb fragment of λ DNA/Hind III fragments (Invitrogen, Carlsbad, CA)) are resolved on a 0.5% agarose gel (20 cm x 20 cm) at 50 V (GNA-200 Pharmacia Biotech). After 16 hr, the DNA is depurinated for 15 min in 0.25 N HCl, denatured 30 min in 0.5 mol/L NaOH/1.5 mol/L NaCl and neutralized for 30 min in 0.5 mol/L Tris, pH 8/1.5 mol/L NaCl. The DNA is transferred for 1 hr to a positively charged nylon membrane (Roche) using a vacuum blotter (Boeckel Scientific, Feasterville, PA). The membranes are spotted at 4 sites with diluted telomeric probe [digoxigenin 3'-end labeled 5'-$(CCTAAA)_3$] (arrows, Figure 1A, B ,C) and then hybridized at 65^{o}C with the probe overnight in 5 x SSC, 0.1% Sarkosyl, 0.02% SDS and 1% blocking reagent (Roche). The membranes are washed 3 times at room temperature in 2 x SSC, 0.1% SDS each for 15 min and once in 2 x SSC for 15 min. The digoxigenin-labeled probe is detected by the digoxigenin luminescent detection procedure (Roche) and exposed on X-ray film. After scanning the LTL signal by densitometry (Figure S1A), the membranes are stripped and re-probed with a molecular weight marker probe (Figure S1B). The superimposition of A and B, using the 4 spotted sites of telomeric probe (arrows), yields the image shown in Figure S1C. In this way, variation in DNA migrations in different lanes minimally affects the analysis.

Table S1. Characteristics of participants with and without leukocyte telomere length measurements at examination cycle 6

	Without LTL measurement* (n=1973)	**With LTL measurement** (n=1143)	p value
Age (years)	60±8.7	60.0±8.6	0.79
Women (%)	1083 (54.9)	604 (52.8)	0.29
Height (cm)	167±9.6	168±9	0.007
Weight (kg)	78.0±17.1	79±17	0.17
Current smoker (%)	310 (15.7)	158 (13.8)	0.17
Systolic blood pressure (mmHg)	129±19	130±19	0.02
Diastolic blood pressure (mmHg)	76±9	76±9	0.52
Treatment for hypertension (%)	569 (28.8)	339 (29.7)	0.66
Diabetes mellitus (%)	186 (9.4)	128 (11.2)	0.13
History of myocardial infarction (%)	64 (3.2)	32 (2.8)	0.56
History of heart failure (%)	15 (0.8)	5 (0.4)	0.39

Data are mean (standard deviation) for continuous traits and n (%) for dichotomous traits.

* In total, 2288 participants did not have LTL measurements done; of these 2288 we excluded those with age <45 years (n=180), AF prevalent at inclusion day (n=72), and missing data at time of inclusion (n=63). Among the 1973 participants without LTL measurement, 339 developed incident AF during follow up.

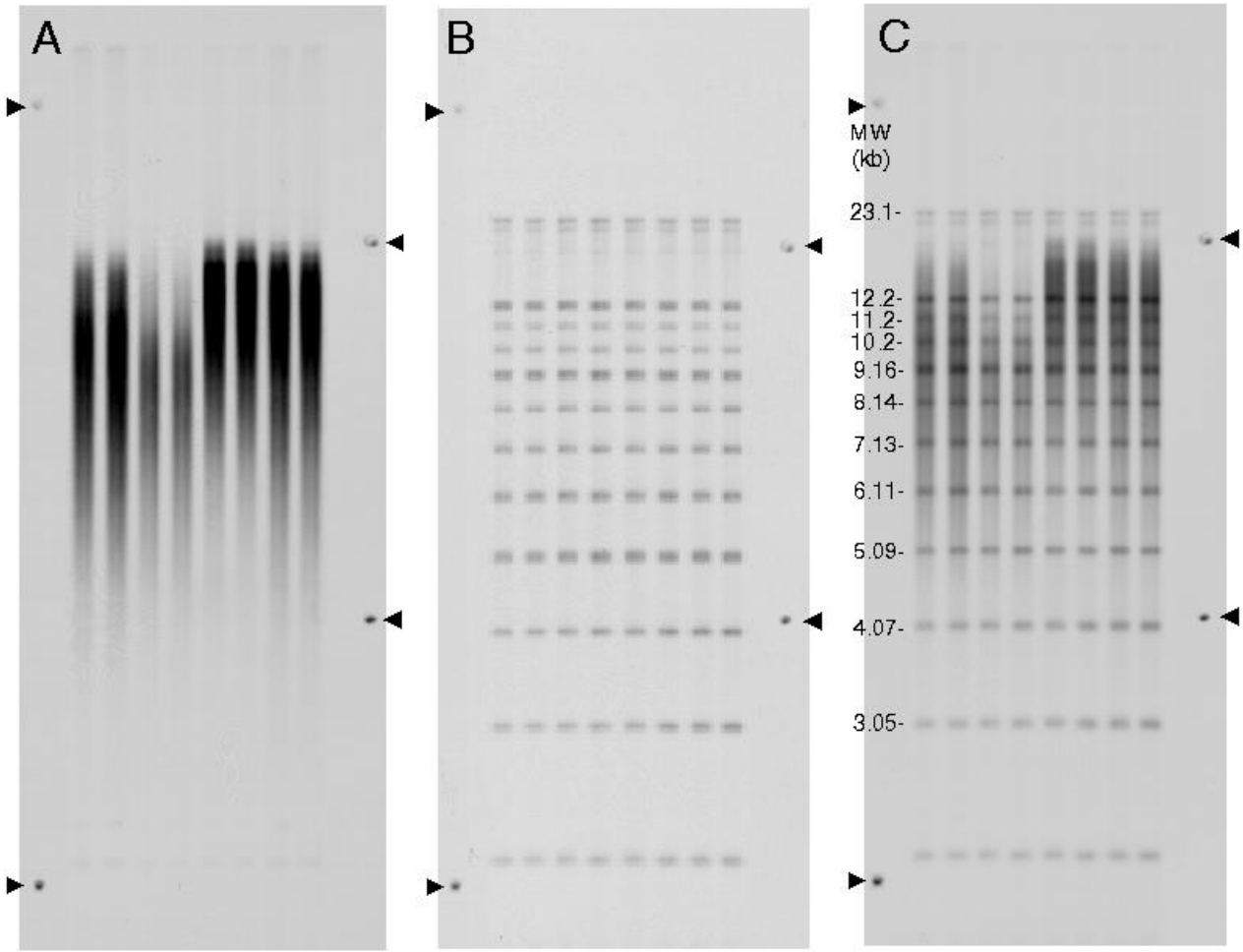
Table S2. Association of leukocyte telomere length and incident atrial fibrillation, when taking the competing risk of death into account

	Age- and sex-adjusted		Multivariable-adjusted*	
	HR (95% CI)†	P value	HR (95% CI) †	P value
Leukocyte telomere length	1.02 (0.87-1.20)	0.78	0.99 (0.84-1.17)	0.92

* Covariates included age, sex, height, weight, smoking, systolic blood pressure, diastolic blood pressure, anti-hypertensive medication, diabetes mellitus, history of myocardial infarction, and history of heart failure.

† Subdistribution hazard ratio associated with a decrease in LTL of 1 standard deviation (0.57 kilobase), estimated with a Fine and Gray model.

Figure S1. Illustration of an autoradiogram showing the terminal restriction fragments (LTLs) (A), the molecular weight ladders (B) and the superimposition of A and B



The position of each band of the MW ladder (y) a is determined by $y= a_0+a_1*\exp^{(-kb/a2)}$.

The mean LTL length is calculated as follows: $LTL = \sum OD_i / \sum (OD_i/MW_i)$, where OD_i is optical density at a given position in the lane and MW_i is molecular weight at that position. This formula accounts for the fact that longer telomeres bind more labeled probe and consequently appear darker on the X-ray film.

Figure S2. Scatter plot of leukocyte telomere length against age at baseline

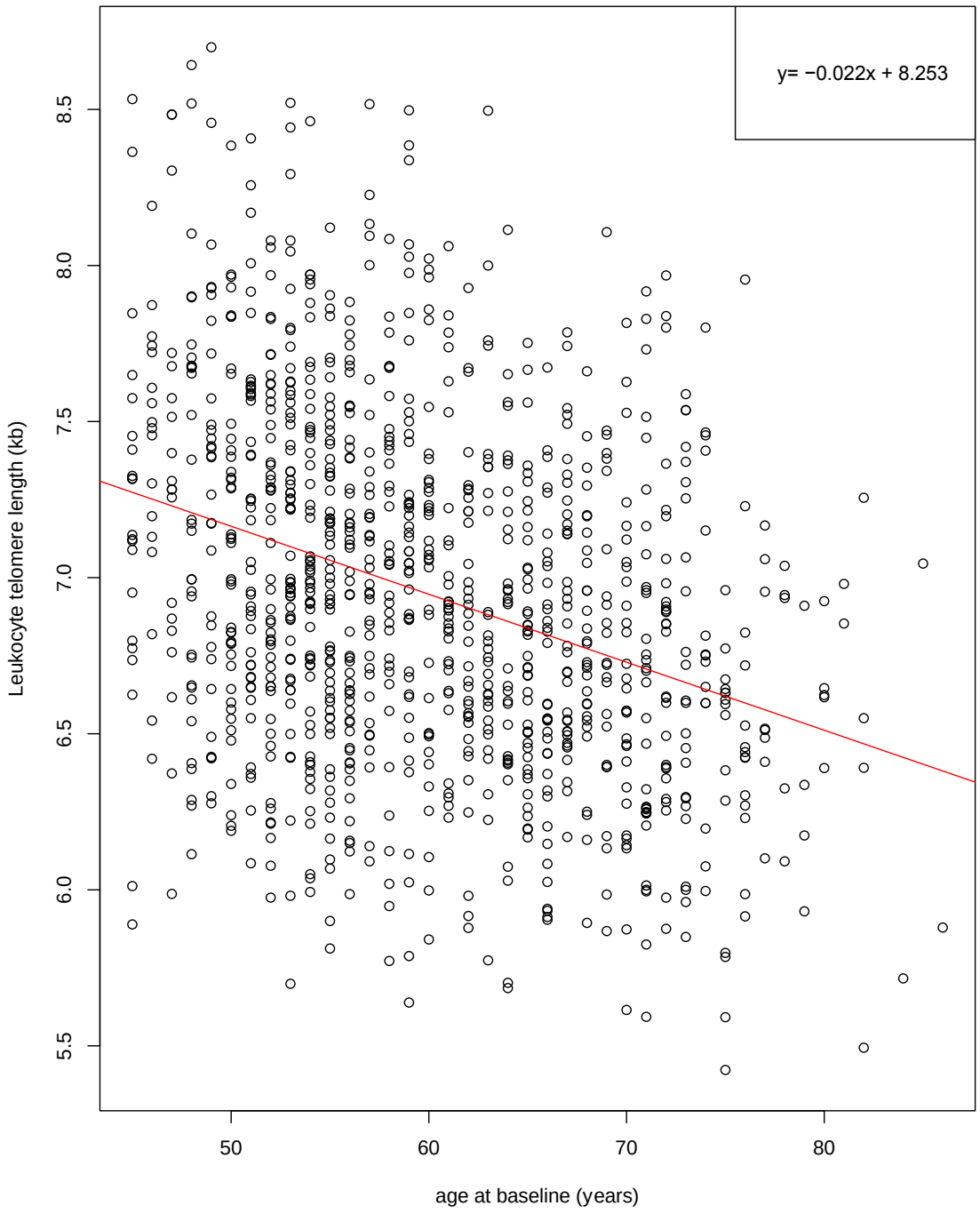


Figure S3. Marginal effect for incident atrial fibrillation associated with a 1 SD decrement in of leukocyte telomere length across the range of age 45-85 years

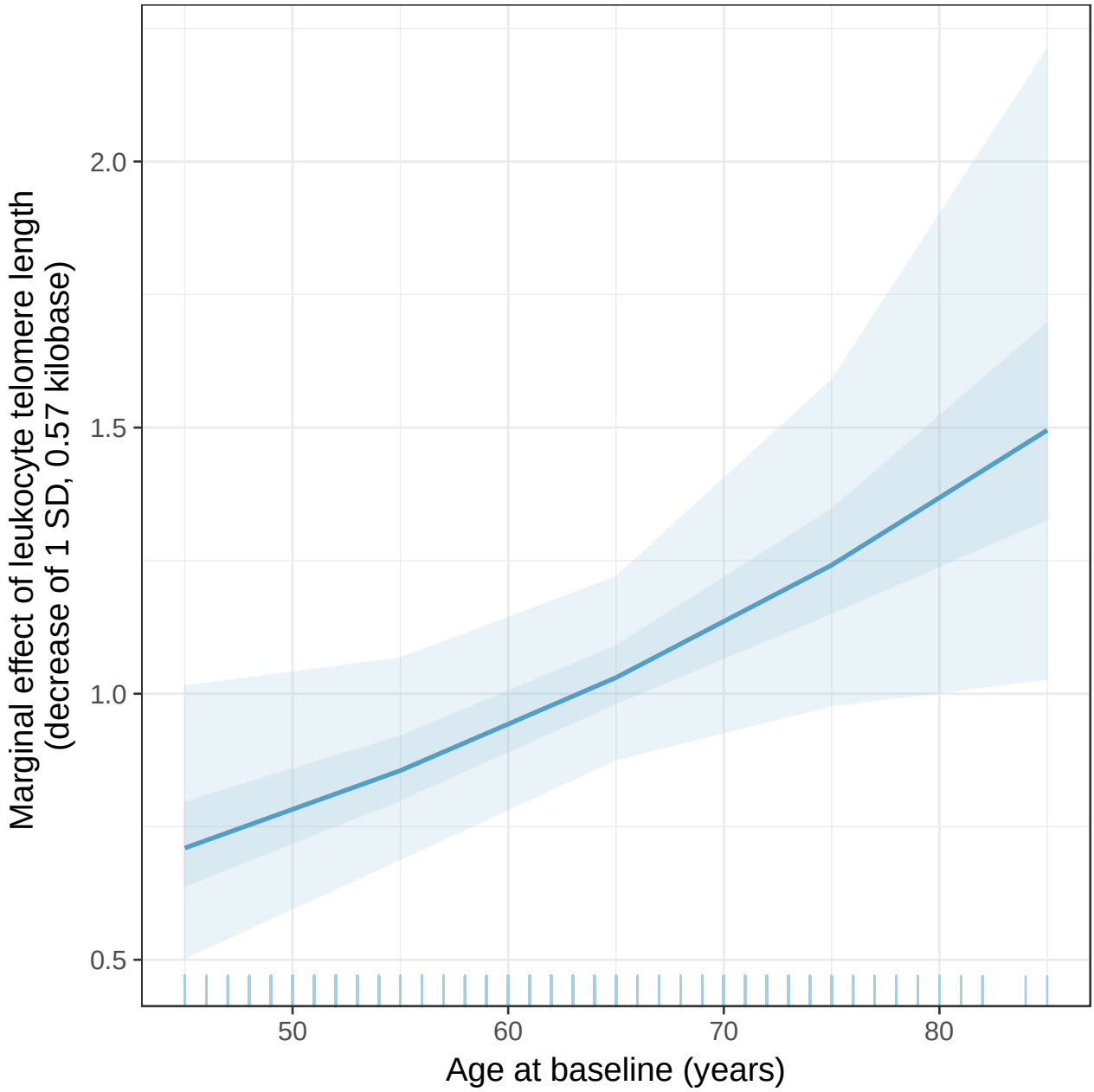
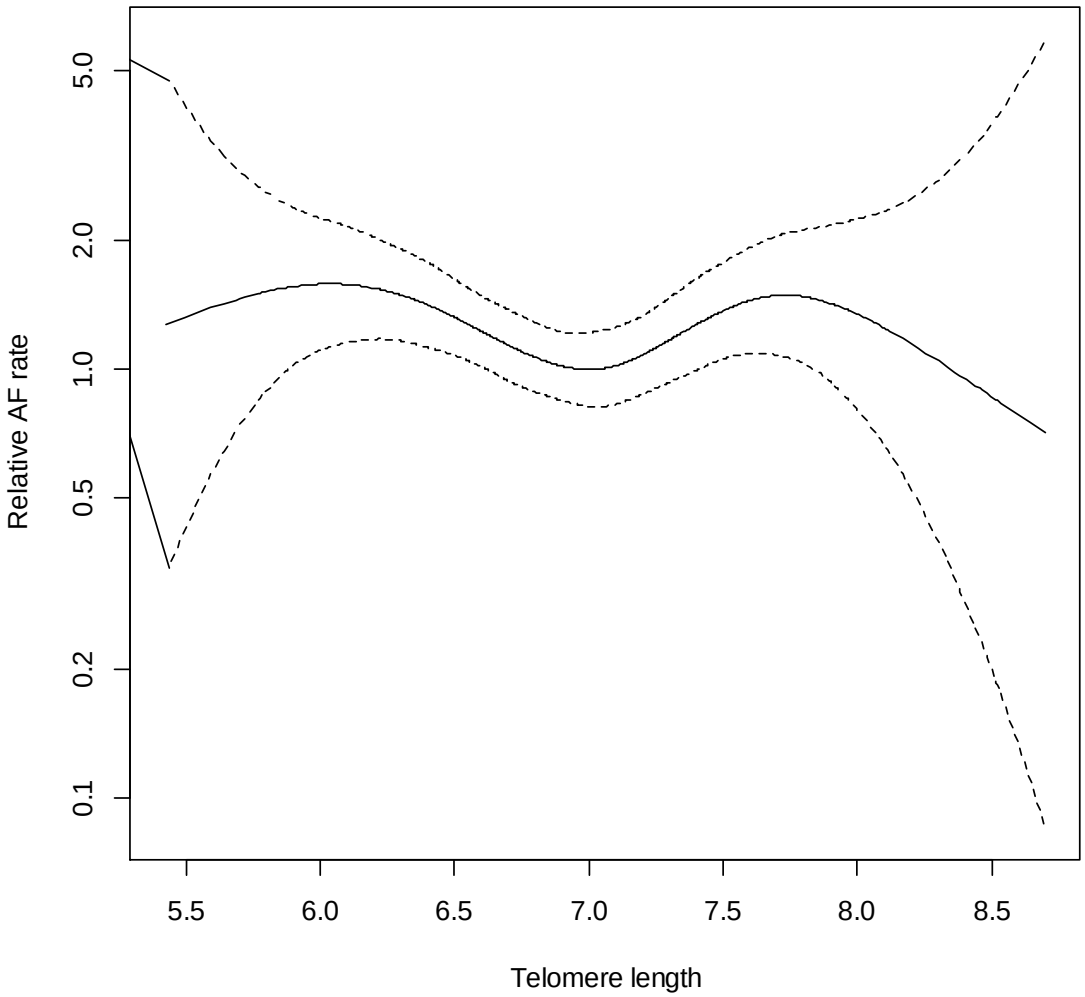


Figure S4. Smoothing spline fit showing the relationship between relative hazard of incident atrial fibrillation and leukocyte telomere length.



Median LTL = 7 was used as reference. p value = 0.15 for a non-linear term