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Genetic Determination of Telomere Size in Humans: A Twin Study of Three Age Groups

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Summary

Reduction of telomere length has been postulated to be a causal factor in cellular aging. Human telomeres terminate in tandemly arranged repeat arrays consisting of the (TTAGGG) motif. The length of these arrays in cells from human mitotic tissues is inversely related to the age of the donor, indicating telomere reduction with age. In addition to telomere length differences between different age cohorts, considerable variation is present among individuals of the same age. To investigate whether this variation can be ascribed to genetic influences, we have measured the size of terminal restriction fragments (TRFs) in *Hae*III-digested genomic DNA from 123 human MZ and DZ twin pairs 2–95 years of age. The average rate of telomere shortening was 31 bp/year, which is similar to that observed by others. Statistical analysis in 115 pairs 2–63 years of age indicates a 78% heritability for mean TRF length in this age cohort. The individual differences in mean TRF length in blood, therefore, seem to a large extent to be genetically determined.

Introduction

The ends of human chromosomes consist of tandem arrays of the (TTAGGG)n repetitive motif (Moyzis et al. 1988). Telomeric repeats are lost during each cell cycle, when small DNA fragments remain uncopied at the discontinuously replicating DNA strand (Olovnikov 1973). In germ cells, this mechanism is compensated by the activity of the telomerase enzyme, a ribonucleoprotein capable of elongating telomeres de novo, thereby overcoming the loss of telomeric repeats (Morin 1989; Blackburn 1991).

Telomere reduction occurs progressively with serial passage of human fibroblasts in culture. Moreover, the initial telomere length of these cells predicts their replicative capacity (Harley et al. 1990; Allsopp et al. 1992). The average telomere size in peripheral blood cells and colorectal mucosa epithelia from older individuals was found to be shorter than that from younger individuals, corresponding with a rate of telomere loss of 33 bp/year (Hastie et al. 1990).

It was proposed that loss of telomeric DNA ultimately leads to cell-cycle exit and senescence (Harley 1991). This may be due to a loss of chromosomal integrity. An increase in terminal chromosomal rearrangements occurs as a function of donor age in human peripheral blood lymphocytes (Bender et al. 1989). This is especially interesting since the subtelomeric regions are extremely gene rich (Saccone et al. 1992). Progressive telomere shortening and accumulation of dicentric chromosomes, which occurs in normal cells in culture, is arrested in a number of immortalized SV40-transformed human embryonic kidney cell lines that express telomerase activity (Counter et al. 1992). Further, the study by Counter et al. indicated that chromosomal aberrations may occur as telomere shortening proceeds beyond a critical length.

Considerable variation of telomere size in peripheral blood lymphocytes from human subjects is present even among individuals of the same age (Hastie et al. 1990). An important question is, therefore, how the individual rate of telomere loss is determined as a function of age. To study the possibility that this can be attributed to genetic influences, the length of terminal restriction fragments (TRFs) was measured in genomic DNA isolated from peripheral blood cells from MZ and DZ human twins 2–95 years of age. The mean TRF length in these samples, as determined by Southern hybridization analysis, decreased 5–10 kb, corresponding to 31 bp/year. Based on statistical analysis and model fitting of the data of 115 twin pairs distributed over three age cohorts (mean ages 4, 17, and 44 years), a 78% heritability for TRF size was found in infants, adolescents, and adults. These results indicate that telomere-size variation is genetically determined to a large extent.

Subjects, Material, and Methods

Study Subjects

Telomere sizes were measured in MZ and DZ twins of four different age groups. Blood samples were obtained from healthy Dutch twins 2–95 years of age, randomly selected from participants of the Netherlands Twin Register (Boomsma et al. 1992, 1993; Snieder et al., in press). The average age was 4.15 years (SD=1.40) in the youngest

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group, 17.1 years (SD=2.40) in adolescent twins, 43.7 years (SD=5.79) in adult twins, and 79 years (SD=7.82) in the oldest group. DNA was isolated from white blood cells as described by I. Meulenbelt, C. J. Williams, G. C. van der Giessen, J. M. te Koppele, and P. E. Slagboom (unpublished data). Twin zygosity was determined by DNA fingerprint analysis (Jeffreys et al. 1985). In the youngest group there were 16 MZ and 15 DZ pairs; there were 15 MZ and 14 DZ adolescent twin pairs and 28 MZ and 27 DZ adult pairs; the oldest group contained 4 MZ and 4 DZ pairs.

Analysis of Telomere Length

Genomic DNA was digested with *Hae*III generating telomeric restriction fragments, which contain the repeat array and a non(TTAGGG) subtelomeric fragment. *Hae*III-recognition sites are absent within the (TTAGGG)n tandem-repeat arrays. Digested genomic DNA (5 μg) was electrophoresed for 1,050 Vh in 0.8%–1% agarose gels containing a maximum of 13 twin pairs per gel (batch) and was subjected to Southern hybridization analysis. Different age cohorts were measured in different batches: the youngest cohort divided in four batches; the adolescent cohort divided in three batches; and the middle-aged cohort divided in eight batches. TRF length measurement by image analysis was done blindly with respect to zygosity of the twins. Eighty percent of the batches contained both MZ and DZ twins.

The Southern blots were hybridized to a random-primed ^{32}P-labeled (TTAGGG)n polymer and to the 33.15 minisatellite core probe (Jeffreys et al. 1985). For preparation of the polymer probe, chemically synthesized $(TTAGGG)_7$ and a complementary oligo were kinased, ligated, and subjected to 20 rounds of a polymerization reaction (95°C, 2 min; 45°C, 2 min; and 74°C, 2 min). The product of this reaction was used as a probe. Hybridization was performed in 7% SDS, 0.5 M NaH_2PO4 pH 7.2, 1 mM Na EDTA at 65°C for 1 h, and the fillers were washed twice in 2.5 × SSC, 0.1% SDS at 65°C for 30 min. Filters were exposed for 3–7 h. For rehybridization, the membranes were stripped for 10 min in 50% formamide, 0.5 × SSC, 10 mM NaH_2PO4 pH 7.2, 25 μg of heparin/ml, 0.5 mM Na EDTA, 0.5% SDS at 65°C and were washed 20 min in 0.1 × SSC, 0.1% SDS.

Autoradiographs exposed within the linear range of signal response were analyzed by computer-assisted image analysis using the ACES (van de Hofstede et al., in press) program adapted for quantification of the smeared telomere hybridization patterns. The position (in kb) of the mean integrated signal was determined.

Statistical Analysis

Genetic analyses were carried out on data from the first three age cohorts (average ages 4, 17, and 44 years). Data from the oldest cohort were excluded from the genetic analysis because there were too few twin pairs in this cohort. To analyze the resemblance between MZ twins, who are genetically identical, and DZ twins, who share 50% of their genes on average, we used a pedigree-based maximum-likelihood method developed by Lange et al. (1988). For a given pedigree of n individuals (n=2 in our case) a vector of observations (x) is defined as well as a vector of expected values $E(x)$ that can depend on measured variables such as age, sex, or batch (gel). In a pedigree, the distribution of of $E(x)$ is expected to be multivariate normal. The expected covariance matrix (Σ) for a pedigree depends both on the relationship between pedigree members and on the genetic model that is specified for the observations. For a given $E(x)$ and Σ, the log likelihood of obtaining the observation vector x is

$$L = -\tfrac{1}{2}\ln|\Sigma| - \tfrac{1}{2}[x-E(x)]'\Sigma^{-1}[x-E(x)] + \text{constant},$$

where the apostrophe (') denotes transpose.

The joint log likelihood of obtaining all pedigrees is the sum of the log likelihood of the separate pedigrees. Estimation involves selection of parameter values under a specific model that maximizes the joint likelihood of all pedigrees. The likelihoods obtained for different models can be compared with the χ^2-difference tests, where $\chi^2 = 2(L_1-L_0)$, and L_1 and L_0 denote the log likelihood for the more general (H_1) and the constrained (H_0) hypotheses (Lange et al. 1976). The df for this test are equal to the number of independent parameters between H_1 and H_0. The FISHER package (Lange et al. 1988) was used for genetic modeling. We first examined the effects of sex, batch within age cohorts, and age regression within age cohorts on the mean phenotype. Next, genetic models that specified twin resemblance to be due to either additive genetic factors or the effects of a shared family environment were tested. Submodels were tested if the effect of these factors was the same in all three age cohorts.

Results

The TRF length as a function of age was assessed by Southern hybridization analysis (fig. 1*A*). The smeared hybridization patterns indicate considerable interchromosomal variation in TRF length as well as intercellular variation in replicative historics. For zygosity determination and as a control for the concentration and quality of DNA and gel, the filters were rehybridized with a minisatellite probe (fig. 1*B*). Telomere size reduction when different age cohorts are compared is illustrated in figure 2. The mean TRF length in white blood cells of 123 twin pairs age 2–95 years corresponded to a loss of 31.0 bp/year ($P \ll .005$; $r=-.71$) (fig. 3). The mean TRF length in the four age cohorts was 8.3 (SD=0.64) for the youngest twins, 7.8 (SD=0.56) for adolescents, 7.3 (SD=0.76) for adults, and 5.6 (SD=0.40) for the oldest subjects. In addition to differences between age cohorts, considerable TRF-length

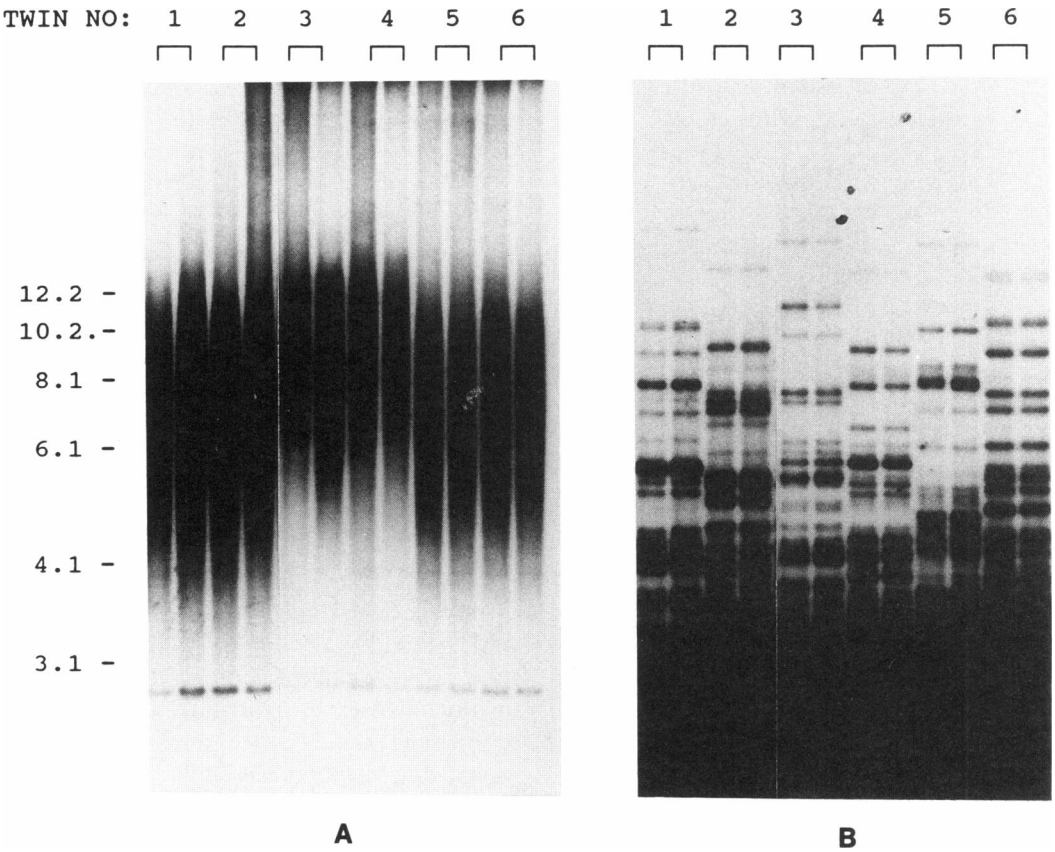

Figure 1 Southern hybridization analysis of MZ twin pairs by the (TTAGGG)n polymer (*A*) and the minisatellite core probe 33.15 (Jeffreys et al. 1985), for zygosity determination and as a control for the quality of DNA and gel (*B*). Size markers (in kb) are indicated. The ages of the twin-pair donors were (1) 19 years of age, (2) 16 years of age, (3) 14 years of age, (4) 14 years of age, (5) 22 years of age, and (6) 21 years of age. The bands appearing at ±2.7 kb are nontelomeric repeat units homologous to the probe.

variation was present within all age cohorts. In general, TRF-length variation among random pairs, i.e., unrelated individuals, was found to be higher than the variation within twin pairs. The variation within MZ twin pairs was found to be the smallest.

Statistical analysis of the data is presented in table 1. Decreases in the log likelihood that are due to deletion of parameters are judged by likelihood-ratio tests. Table 1 shows the different models that were evaluated, their log likelihoods, and the χ^2 test statistics used to compare the goodness of fit of the more restricted models versus the more general model. Twice the difference between the log likelihoods of these models is distributed as χ^2. The most general model both allowed for the effects of sex, batch, and age regression within cohorts on the phenotype and estimated six different correlations for MZ and DZ twins in the three age cohorts. Differences between age cohorts explained 18% of the variance in TRF size. Batch effects within the three cohorts explained 42% of the total variance. Estimates for twin correlations from the general model are shown in table 2. For the part of the phenotypic variance that was not explained by cohort or batch, the pattern of estimated twin correlations suggests that individual differences in TRF size are to a large extent genetically determined. There are no significant effects of sex or age regression within cohorts on the mean telomere size, as indicated by models B and C in table 1. Model E indicates that MZ and DZ correlations were not significantly different in the three cohorts. Model F is a test of the additive genetic model, in which the MZ correlation is exactly twice the DZ correlation. This model fits the data as well as model E, in which MZ and DZ correlations were estimated separately. Estimates for MZ and DZ correlations were .776 (standard error [SE]=0.046) and .388 (SE=0.023). Model G tests whether the MZ and DZ correlations may be equated to each other; this is a test of the shared-environment model. It can be seen from the large decrease in likelihood that shared family environment cannot explain twin resemblance as well as an additive genetic model can. Heritability based on the final model is 78% in all three cohorts. A plot of cohort- and batch-corrected MZ and DZ correlations is presented in figure 4. In this figure the TRF size of the firstborn member of a twin pair is plotted against that of the second born, clearly illustrating the larger resemblance of TRF sizes of MZ twins, as compared with DZ twins.

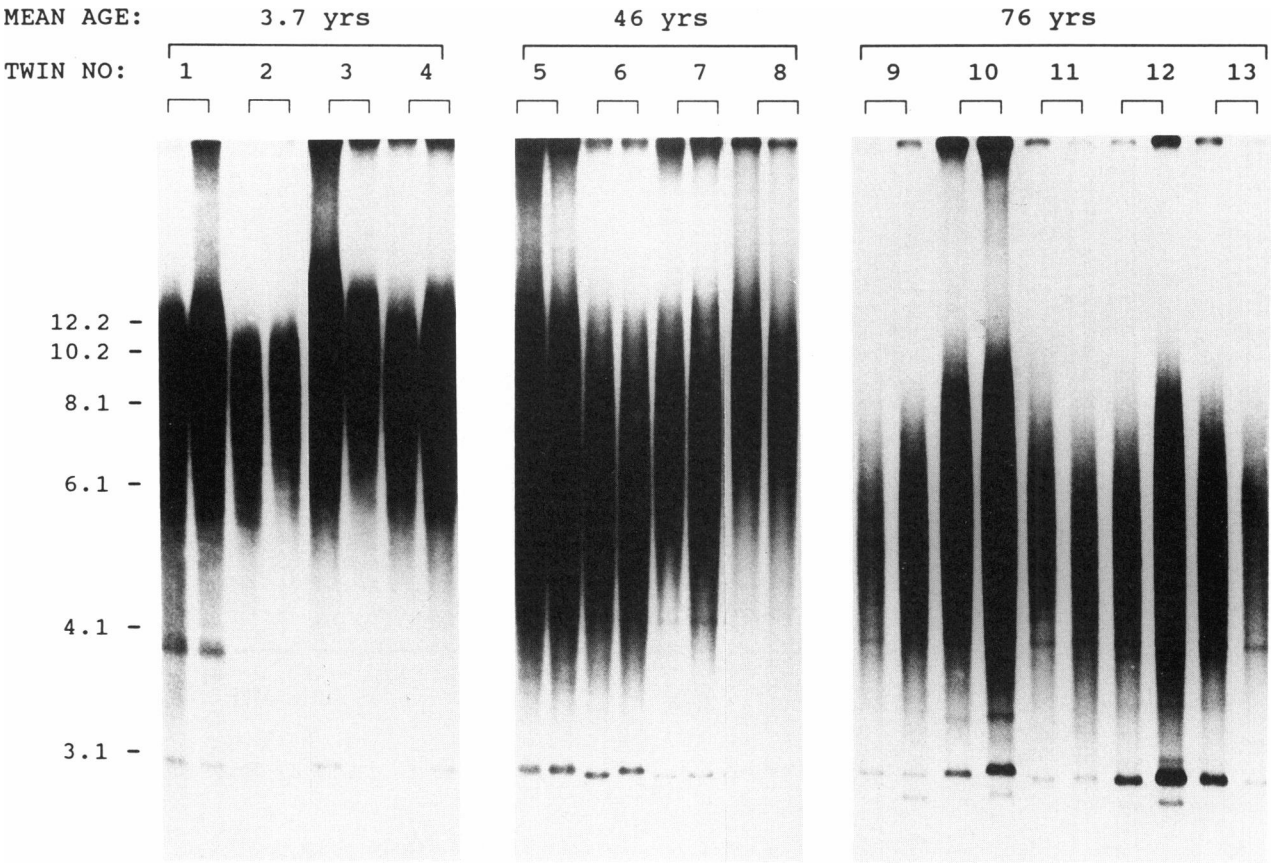

Figure 2 Southern hybridization analysis of a subset of twin pairs of different age groups, using the (TTAGGG)n polymer as a probe. Average donor age and size markers (in kb) are indicated. All twin pairs were MZ except for pairs 8, 9, 11, and 13, who were DZ.

Discussion

The most striking finding of our study is that individual differences in mean TRF size in genomic DNA isolated from white blood cells are to a large extent genetically determined. Heritability based on model fitting of the data is 78% in 4-, 17-, and 44-year-olds. With regard to the fact that the total variance also includes error variance (heritability = genetic variance/total variance), the high heritability suggests that individual differences are almost entirely genetic in origin.

Considerable differences were observed between the means of assay batches (gels). This could be due either to unnoticed variations in electrophoretic conditions among different gels (although the amount of Vh was equal for all gels) or to concentration differences between DNA sam-

Table 1

Genetic Model Fitting by Using FISHER (Lange, et al. 1988)

Model	Likelihood	Tested Against	df	χ^2
A (Full model)[a]	59.101	...	...	...
B (No sex differences)	58.581	A	1	1.040
C (No age regression within cohorts)	58.500	B	1	.162
D (No batch effect within cohorts)	9.773	C	12	97.454[b]
E (MZ correlation equal across cohorts and DZ correlation equal across cohorts)	54.447	C	4	8.106
F (Additive genetic model: r(MZ) = 2r(DZ)	54.413	E	1	.068
G (Shared environment model: r(MZ) = r(DZ)	47.792	E	1	13.310[b]

[a] Batch and sex effects, age regression, and six twin correlations.
[b] Significant deterioration in fit.

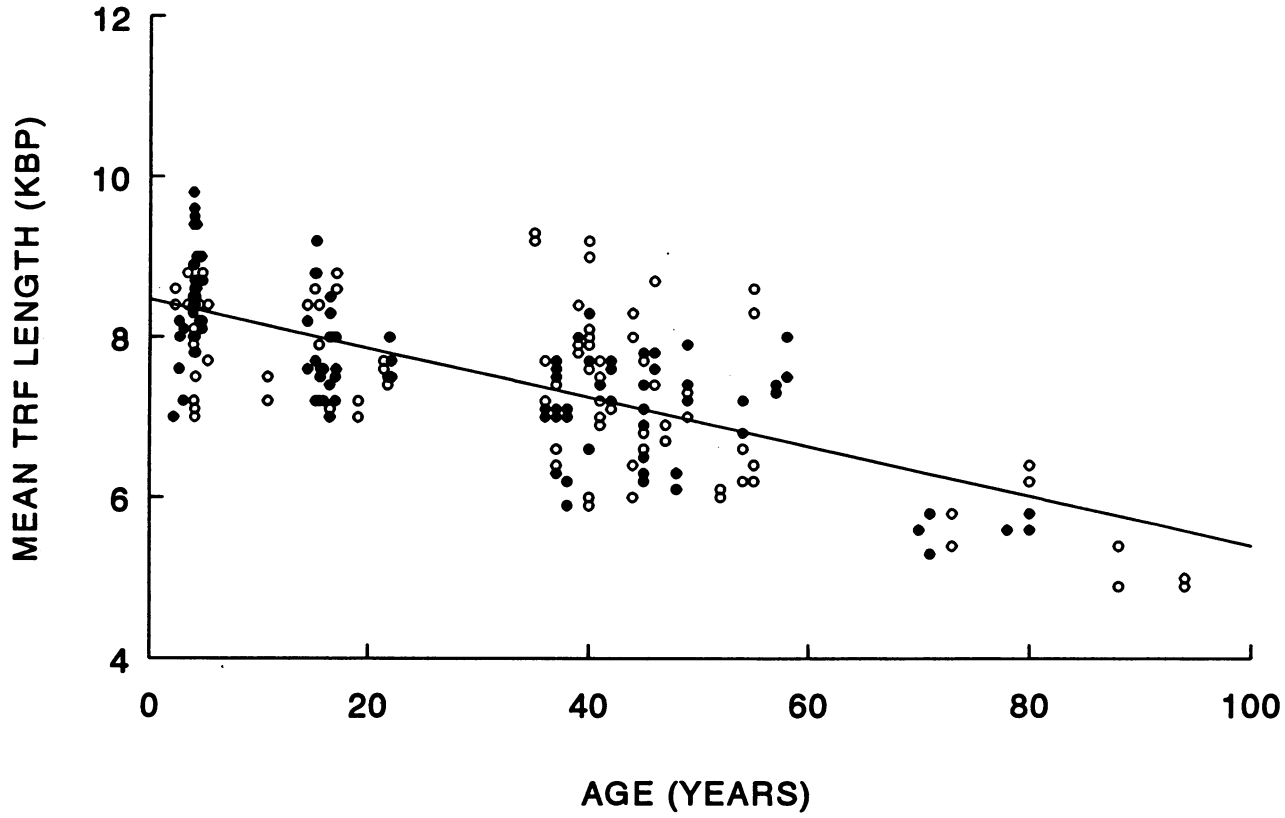

Figure 3 Mean TRF length as a function of donor age, as measured in genomic DNA from peripheral blood lymphocytes, as described in Subjects, Material, and Methods. Each data point represents measurement on a single gel. A blackened circle represents a DZ twin, and an unblackened circle represents an MZ twin. The slope (−31 bp/year) of the linear regression line is significantly different from 0 ($P \ll .005$; $r = -.71$).

ples (with the smallest variation among samples on a single gel) and, therefore, between autoradiographic signals. By using different exposure times, we have measured each DNA sample within the linear range of signal response of the X-ray films. Possibly, the quantification of smeared autoradiographic patterns and estimation of the midpoint position of the integrated signal are most accurately determined if all samples involved in the experiment have equalized signal intensities. The best way around a batch effect would be a randomization of all individuals in the study, across all batches. For comparison of the DNA fingerprint patterns in the zygosity test, however, twin-pair samples were run on the same gel.

Table 2

Maximum-Likelihood Parameter Estimates (SEs) of Twin Correlations from the Full Model

	Correlations Free
MZ 4 years	.562 (.187)
DZ 4 years	.321 (.132)
MZ 17 years	.853 (.055)
DZ 17 years	.665 (.136)
MZ 44 years	.833 (.048)
DZ 44 years	.496 (.163)

The differences in mean TRF size among individuals of the same age cohort could reflect (i) interindividual TRF length differences in the germ line as were observed by Allsopp et al. (1992); (ii) interindividual differences in turnover rate of white blood cells in vivo; or (iii) interindividual differences in the amount of telomeric DNA lost per cell cycle. To distinguish between these possibilities, the individual rate of telomere loss in vivo should be measured longitudinally and, preferably, both in subpopulations of white blood cells and in sperm cells. TRF length may predict the replicative potential of the somatic cells of an individual (Allsopp et al. 1992). Furthermore, the high heritability of TRF length in humans, as indicated by our data, argues against high rates of somatic recombination as were found for the hypervariable subtelomeric and telomeric regions of inbred mice (Starling et al. 1990).

The genetic basis for individual TRF-length variation may be found in the presence of telomerase gene variants with variable activity in germ-line and/or stem-cell pools. Alternatively, our findings may be explained by a genetically determined individual variation of cell turnover rate and/or immunological response to antigenic exposure. The increased rate of telomere loss in Down syndrome pa-

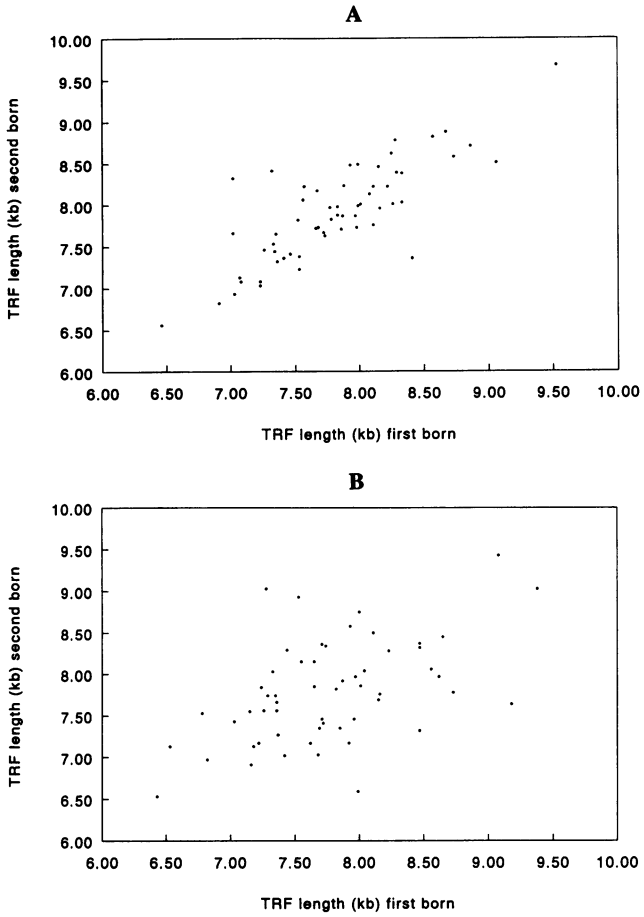

Figure 4 TRF sizes, corrected for cohort and batch effect (based on maximum-likelihood analysis using FISHER), in 59 MZ (*A*) and 56 DZ (*B*) twins. Plot of values is of firstborn versus second-born twin.

tients (Vaziri et al. 1993) could indicate the involvement, in telomere length, of genes on chromosome 21. Genetic control has also been suggested to explain strain-specific variations in telomere size in mice (Kipling and Cooke 1990). The length and stability of yeast telomeres are controlled by at least four genes (Carson and Hartwell 1985; Lundblad and Szostak 1989). Interestingly, a mutation in one of these loci, the *est*1 locus, causes gradual loss of telomeric repeats, aneuploidy, and senescence (Lundblad and Szostak 1989).

Telomere shortening in mitotic tissues might eventually lead to chromosomal aberrations and expression changes of genes located in subtelomeric regions (Biessmann and Mason 1992), which may affect cellular functions. Data on transformed human fibroblasts indicate that instabilities of the telomeric regions and cell death occur when a critical minimum telomere length of 1.5 kb (TRF length of 4 kb) is passed (Counter et al. 1992). Telomere loss in vivo, as measured in blood DNA of random individuals, occurs on average at a rate of 31–45 bp/year (Hastie et al. 1990; Vaziri et al. 1993; present study). Whether essential coding sequences are affected during an individual's life span would depend on the individual rate of telomere loss. Mean TRF lengths <5 kb were not observed in any of the studies including old individuals (Hastie et al. 1990; Vaziri et al. 1993; and the present study). It cannot be discounted that the relatively small number of aged individuals investigated thus far represents a selected sample. The large variation of mean TRF length among the younger individuals in our study indicates that, if telomere loss occurs continuously, TRF lengths <4 kb are expected to be reached at old age in at least some of these individuals. Telomere shortening may well be involved in cell-cycle control and cellular senescence. Our data indicate that such a process in vivo is largely determined by genetic influences.

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