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## Report

# Mapping of a Major Locus that Determines Telomere Length in Humans

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**Telomere length is a crucial factor for both normal chromosomal function and senescence. Mean telomere length in humans shows considerable interindividual variation and strong genetic determination. To see if a locus (or loci) affecting telomere length in humans could be mapped, we performed a quantitative-trait linkage analysis of mean leukocyte telomere-restriction–fragment (TRF) lengths, measured by Southern blotting, in 383 adult subjects comprising 258 sib pairs. Heritability of mean (±SE) TRF was 81.9% ± 11.8%. There was significant linkage (LOD score 3.20) of mean TRF length to a locus on chromosome 12, which explained 49% of the overall variability in mean TRF length. We present preliminary analysis of a strong candidate gene in the region, the DNA helicase *DDX11*. In conclusion, we report mapping of the first locus that determines mean telomere length in humans. Identification of the gene involved and elucidation of its mechanism of action could have important implications for our understanding of chromosomal assembly, telomere biology, and susceptibility to age-related diseases.**

Telomeres are special functional complexes, at the ends of eukaryotic chromosomes, involved in maintaining genetic stability and in regulation of cellular life span (Blackburn 2001; Blasco 2003). Telomere homeostasis is relevant to normal aging and a wide range of disease states, including cancers and age-related disorders (Blackburn 2001; Blasco 2003). Telomeres are made up of a variable number of tandem repeats (TTTAGGG in humans) that extend over several thousand base pairs. Telomere lengths are characteristic in each human individual (Takubo et al. 2002) but show wide interindividual variability (Slagboom et al. 1994; Jeanclos et al. 2000; Takubo et al. 2002). Heritability of mean telomere length in leukocytes has been estimated at 78%–84%, on the basis of twin and family studies (Slagboom et al. 1994; Jeanclos et al. 2000). In proliferation of somatic cells that lack telomerase, telomeres progressively shorten because of the end-replication problem of linear DNA molecules; telomere length has emerged as an important determinant of replicative senescence and cell fate (Allsopp et al. 1992), possibly by its effect on telomere capping (Blackburn 2000). Telomere length therefore reflects aging, and shorter telomeres have been associated with a variety of age-related diseases (Blackburn 2001; Blasco 2003; Serrano and Andres 2004). Although a locus that determines telomere length in some mouse strains has been mapped to chromosome 2 (Zhou et al. 2000), the nature of the genetic determinants of telomere length in humans remains unclear.

To see if a locus (or loci) affecting telomere length in humans could be mapped, we performed a quantitative-trait linkage analysis of mean leukocyte telomere-restriction–fragment (TRF) lengths, measured by Southern blotting, of 383 adult subjects (291 males, 92 females) from 173 families comprising 258 sib pairs (table A1 [online only]). The mean age (±SD) of the subjects was 65.8 ± 6.4 years (range 47–82 years). As was found elsewhere (Benetos et al. 2001; Brouilette et al. 2003; Nawrot et al. 2004), there was an age-related decrease in mean TRF length (±SE) in both men (29.9 ± 5.6 bp/year) and women (16.8 ± 9.9 bp/year) (fig. 1*A*). The difference in rate of decline between the sexes was not significant ($P = .61$). However, consistent with previous data (Benetos et al. 2001; Nawrot et al. 2004), the mean age-adjusted TRF length in women was shorter than in

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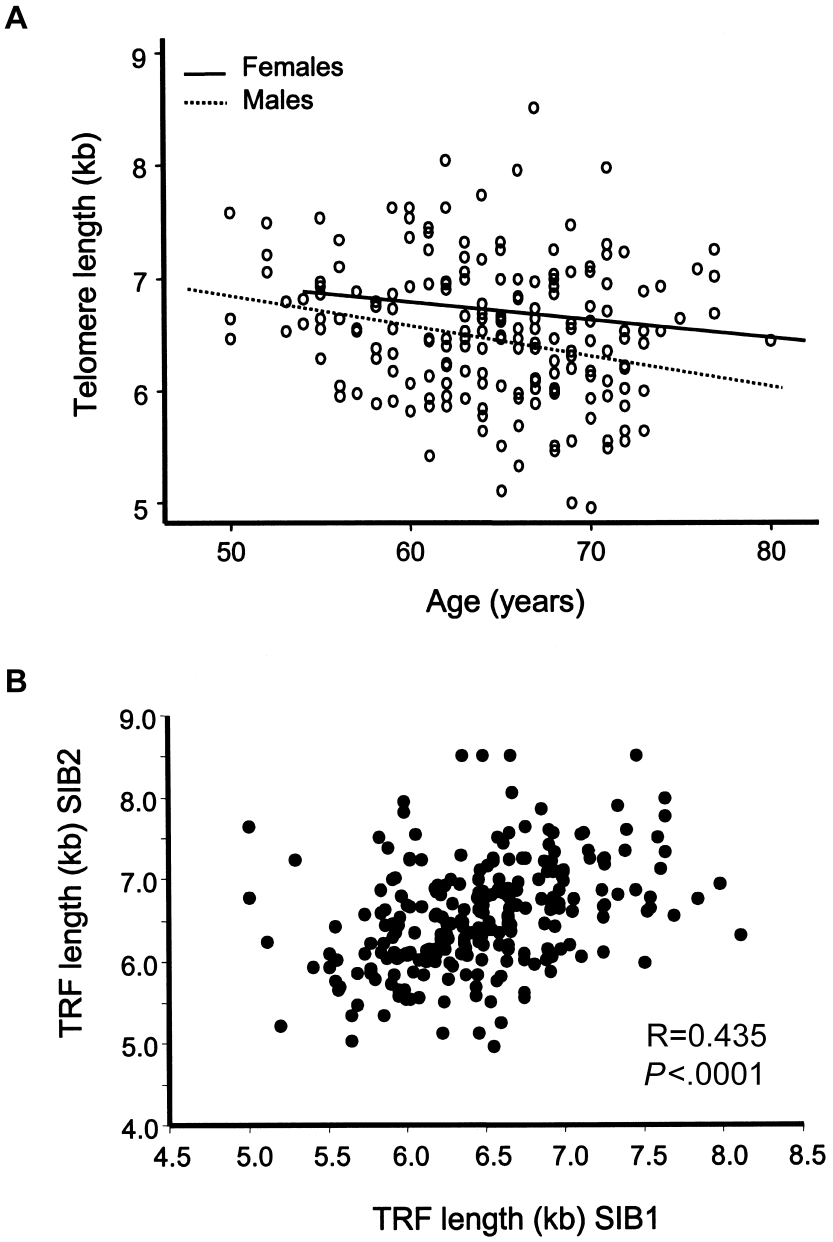


**Figure 1** Age relationship and intersibling correlations for mean TRF length. *A,* Each subject's mean TRF length, plotted as a function of the subject's age. Note the high interindividual variability in mean TRF length at any age. The regression lines show the mean decrease in TRF length with age of females (*solid lines*) and of males (*dotted lines*). The coefficient of variation in males was 9.6% and in women was 8.8%. *B,* The correlation in mean TRF length (unadjusted) between sib pairs.

men (difference [± SE] 271.5 ± 64.7 bp; $P < .001$). There was a highly significant intersibling correlation in TRF length (fig. 1*B*). Under the assumption that the entire familial resemblance in telomere length is genetic, a heritability index (h2) (± SE) of 81.9% ± 11.8% was obtained for mean TRF length. This is similar to previous estimates (Slagboom et al. 1994; Jeanclos et al. 2000). Age ($P < .001$) and sex ($P < .001$) were significant covariants, together accounting for 8.7% of the variance in the trait, and were included in the linkage-analysis model to allow an appropriate adjustment.

An initial genome scan with 400 microsatellite markers—at intervals of ~10 cM (ABI-Prism Linkage Mapping set, MD-10 panels version 2.5), with an average heterozygosity of 0.79—identified significant linkage to chromosome 12 (fig. 2*A*). A maximum two-point LOD score

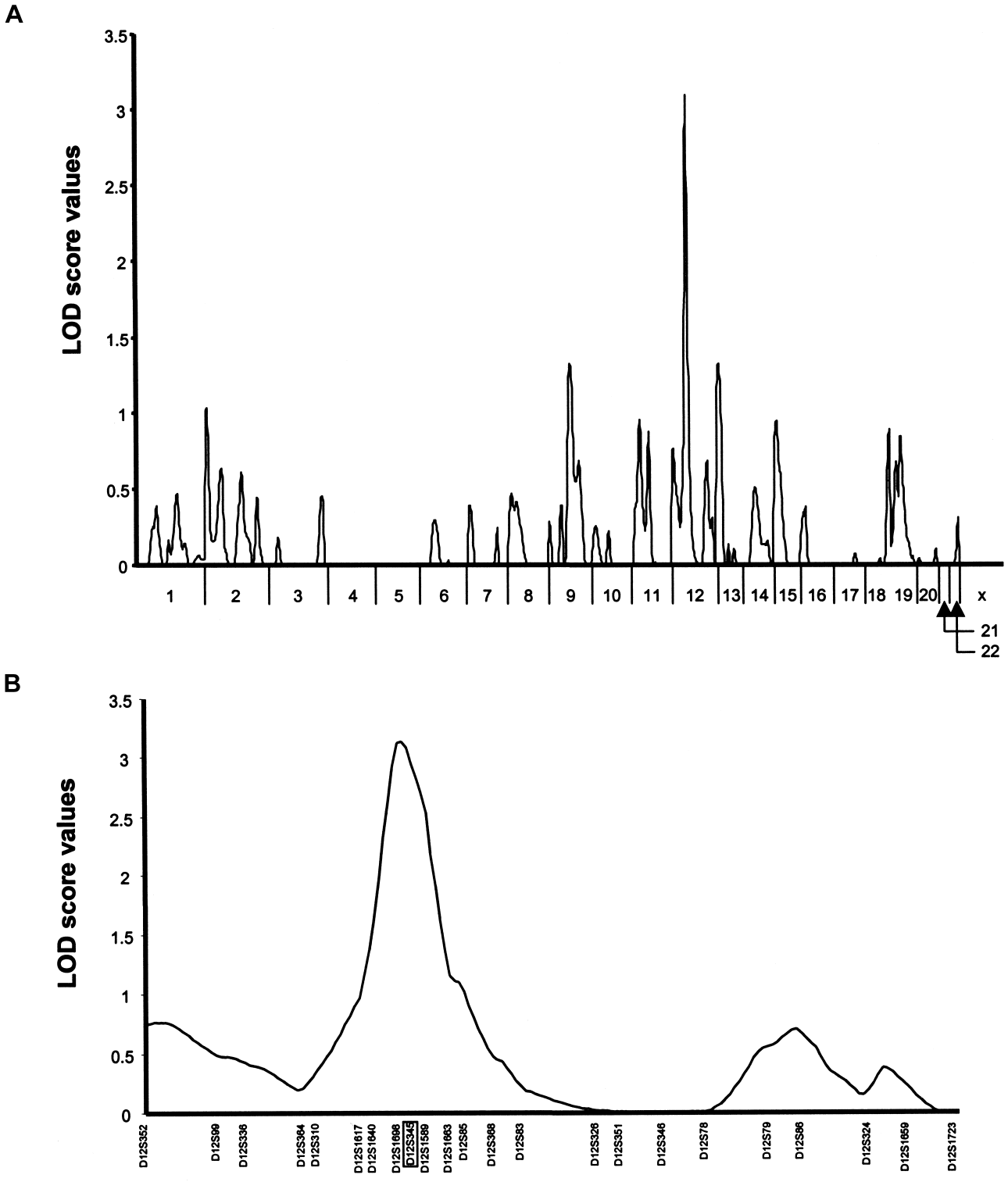


**Figure 2** Results of genome-scan analysis for mean TRF length. *A*, The peaks and corresponding LOD scores obtained on each chromosome from multipoint linkage analysis performed using SOLAR (see the "Methods" section of appendix A [online only] for details). *B*, A more detailed representation of the linkage observed on chromosome 12, with results from the additional markers typed in the region of the observed peak.

of 3.21 was obtained for marker *D12S345,* by use of the Sequential Oligogenic Linkage Analysis Routines (SOLAR) package. Four additional markers in the region were typed to confirm linkage and for fine mapping of the genetic interval. Marker *D12S168* showed a LOD score of 3.03, and multipoint analysis showed a maximum LOD score of 3.20 between markers *D12S1640* and *D12S1589* (fig. 2*B*). Analysis by use of the Multipoint Engine for Rapid Likelihood Inference (MERLIN) program gave similar results (LOD score 3.07, $P = .00008$ at marker *D12S345;* LOD score 3.04, $P = .00009$ at marker *D12S1698*). Using MERLIN, we made 1,500 simulated genome scans under the same conditions used during the analysis (family structure, phenotype, marker spacing, allele frequencies, and missing-data patterns). Sixty-seven simulations gave two consecutive markers with a LOD score >3.0. This equates to a genomewide *P* value of .044 for our finding. Heritability analysis showed that 49% of the total interindividual variability of mean TRF length could be attributed to the locus on chromosome 12. For the remainder of the heritable influence on mean TRF length, the likely scenario is that this influence is the result of several genes with smaller effects, although our findings cannot exclude the possibility that another major locus is responsible. We observed peaks with LOD scores of 1–1.5 on chromosomes 2, 9, and 13 (fig. 2*A*) that could harbor such loci, but larger studies are necessary to confirm this. Our findings, however, do not support the recent proposal—which is based on patterns of correlations within nuclear families—that the majority of inheritance of telomere length is X linked (Nawrot et al. 2004).

A feature of our study that requires comment is the measurement of mean TRF length in sib pairs with coronary heart disease (CHD) (see the "Methods" section of appendix A [online only]). This choice was pragmatic: the initial genome-scan data were already available for these subjects. Because we (Brouilette et al. 2003) and others (Cawthon et al. 2003) have found an association between shorter telomeres and risk of CHD, the question arises as to whether this association could have affected our findings. We believe that this scenario is unlikely, because the subjects in the study form a relatively homogeneous sample of patients with CHD. Selection into the study from within the population of patients with CHD is unlikely to be related either to telomere length or to genotype. Furthermore, multipoint linkage analysis within the sample with CHD as the phenotype showed no evidence of significant or even suggestive linkage for any of the telomere-length QTL peaks. Therefore, although the findings need to be confirmed in patients unaffected by CHD, there is every reason to suppose that our findings would generally apply

According to the Ensembl database, the 1-LOD interval for the locus on chromosome 12 spans 13.2 Mb and contains 34 genes (42, if isoforms and other predicted transcripts are included) (table A2 [online only]). Among these 34, the DNA helicase *DDX11* (MIM 601150) immediately emerged as a strong positional candidate to explain the effect on telomere length. Helicases unwind double-stranded DNA and RNA and are involved in a wide range of chromosome-related functions, including transcription, replication, segregation, and DNA repair. Many helicases have roles in maintenance of telomeres and in telomere-length control. These include the RecQ helicase involved in Werner syndrome in humans, a disorder associated with premature aging (Yu et al. 1996), and the *Sgs1* and *Pif1* helicases in yeast (Zhou et al. 2000). The yeast homologue of *DDX11, Chl1,* is involved in chromosome transmission and normal cell-cycle progression, with mutants exhibiting a senescent phenotype (Amann et al. 1997). The *DDX11* gene spans 31 kb and comprises 27 exons. The gene is highly polymorphic, with >350 SNPs already described in the SNP Consortium Database. Analysis of SNPs in the region typed in the HapMap Project suggested that the *DDX11* gene lies within a 61-kb linkage-disequilibrium block with two major haplotypes (see the "Methods" section of appendix A [online only]). In a preliminary analysis, we examined the association of five SNPs that spanned the entire *DDX11* gene (including three tag SNPs for the common haplotypes and two additional SNPs to cover the 3′ end of the gene) (table A3 [online only]) with mean TRF length in our subjects. To gather data from independent chromosomes for this analysis, one individual randomly selected from each family ($n = 173$) was initially studied. The results confirmed that the *DDX11* gene is in a linkage-disequilibrium block. The block is defined by two principal haplotypes—hap A: AGGCA (frequency 0.445) and hap B: TCATG (frequency 0.536)—at the SNPs shown in table A3 (online only). However, the mean TRF length, adjusted for age and sex, was not significantly different between the *DDX11* genotype groups defined by the two haplotypes (AA: 6.47 ± 0.50 kb [±SD]; AB: 6.48 ± 0.60 kb; BB: 6.60 ± 0.61 kb; $P = .43$). To include all the information available, we also genotyped the remainder of the cohort and analyzed the full data ($n = 383$) with a random-effects model, taking familial correlations into account. This analysis was also nonsignificant ($P = .28$). These findings suggest that the *DDX11* haplotypes defined by these SNPs do not have a large effect on mean TRF length nor solely explain the linkage observed. However, the results do not exclude an involvement of *DDX11* in telomere-length determination, which will require a more detailed determination of the extent of variation in the gene. This work is currently ongoing, as is the study of other possible candidate genes in the region.

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## Electronic-Database Information

The URLs for data presented herein are as follows:

Ensembl, http://www.ensembl.org/ (for genetic distance between markers and identification of genes in the interval of interest)
HapMap Project, http://www.hapmap.org/ (for SNP information)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *DDX11*)
SNP Consortium Database, http://snp.cshl.org (for SNP information)

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