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Epigenetic inheritance of telomere length obscures identification of causative *PARN* locus

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Mean leucocyte telomere length (LTL) has been associated with cancer and several age-associated diseases. Common variants within or near genes known to be involved in telomere biology (*TERT*, *TERC*, *RTEL1*) have been associated with mean LTLs in genome-wide association studies (GWAS).¹² Rare variants in these genes have also been found to cosegregate with short telomere lengths and lung disease in familial pulmonary fibrosis kindreds.^{3–5} Rare variants in an additional telomere-related gene, *PARN* (polyadenylation-specific ribonuclease deadenylation nuclease), were recently found by exome sequencing older adults with familial pulmonary fibrosis³ as well as patients with dyskeratosis congenita, a disorder of telomere biology generally affecting children.⁶⁷ *PARN* has not been identified by GWAS studies as a contributor of human telomere length regulation. Here we determine if the *PARN* locus could have been identified using genome-wide linkage analysis of LTLs in one large familial pulmonary fibrosis kindred that has a cosegregating *PARN* nonsense mutation.

This study was approved by the institutional review board at the University of Texas Southwestern Medical Center. Written informed consent was obtained from all available family participants, who were recruited from 2006 to 2010 as previously described.³ A heterozygous loss of function *PARN* mutation (c.529C>T, Gln177*) cosegregates with pulmonary fibrosis in the kindred shown in figure 1A. The proband (III:10) died at the age of 43 of complications of progressive pulmonary fibrosis; his mean LTL (as measured by a QPCR assay³) was <1st percentile as compared with age-matched controls. His paternal aunt (II:8) also died of pulmonary fibrosis and her mean LTL was <10th percentile. In this kindred, there are 11 individuals with pulmonary fibrosis or LTLs <1st percentile, suggesting a sufficient number of affected individuals for a linkage study. All four

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individuals who died from progressive pulmonary fibrosis (II:6, II:8, II:9 and III:10) inherited a heterozygous *PARN* nonsense mutation (Gln177*). Seven related family members ranging from 28 to 80 years of age have LTLs <1st percentile. Overall, age-adjusted telomere lengths of non-mutation carrier relatives are longer than those of the *PARN* mutation carriers but shorter than those of the non-related spouses (figure 1B).

Genomic DNA of 23 individuals from this family was genotyped using the Illumina HumanCytoSNP-12 BeadChip SNP panel of approximately 220 000 markers. Call rates varied from 98.9% to 99.9%. We chose 6915 SNPs to construct a linkage map across the autosome with spacing of approximately two SNPs per centimorgan. An affected-only analysis approach was employed and three classification schemes were considered; individuals with pulmonary fibrosis and individuals with a blood LTL below different cut-offs (1st, 5th or 10th percentile) were assigned an 'affected' status and all others were assigned an unknown affection status. Multipoint linkage analysis was carried out under a dominant model with a penetrance of 0.9, a sporadic rate of 0.001 and a disease predisposing allele frequency of 0.001 using MORGAN.⁸ Figure 1C shows the linkage results of chromosome 16 relative to the location of the *PARN* gene (indicated by the arrow). Classifying those with pulmonary fibrosis or a telomere length <1st percentile as affected led to the highest linkage peak, with a logarithm (base 10) of odds (LOD) score of 2.6 (p value 2.5×10^{-3}). There is no other region across the genome with an LOD score >1.0. The linked region does not encompass the *PARN* gene as its telomeric border misses the gene by 4.5 Mb. The 'misclassification' of two family members (III:2 and IV:2), who inherited the wild-type *PARN* allele and who have LTLs <1st percentile, as affected individuals led to the shifting of the linkage peak away from the *PARN* locus.

Although unlikely, it is possible that another genetic regulator residing within the linkage peak has a greater role than the *PARN* nonsense allele in regulating telomere length in this family. Alternatively, as *PARN* regulates post-transcriptional modification and stability of multiple different mRNAs, snoRNAs and TERC,⁶⁷⁹ the effects of *PARN* mutations on telomere length are likely indirect and may lead to telomere shortening that less perfectly cosegregates with heterozygous mutations. This kindred includes members who have short telomeres and carries a heterozygous *PARN* mutation as well as members who have short telomeres without carrying the same mutation. Short telomeres in non-mutation carriers might be related to 'epigenetic' transmission of short telomeres in the presence of a normal *PARN* allele. The effects of both genetic and epigenetic inheritance of LTLs seen in this family have also been demonstrated across multiple different kindreds with mutations in other telomere-related genes.³¹⁰ It is currently unclear if the epigenetic effect of telomere shortening is sufficient to lead to the phenotype of progressive pulmonary fibrosis in the absence of an inherited heterozygous *PARN* mutation.

Disentangling the effects of genetic variants from epigenetic and environmental influences on telomere length can be challenging in population studies. This study demonstrates that epigenetic effects of inherited telomere lengths can be quite sizable. Short telomere lengths can be inherited independent of a segregating deleterious mutation in a telomere-related gene and can lead to the grouping of individuals both with and without a causative monogenic mutation into the same group with an extreme phenotype (such as an LTL of

<1st percentile). The *PARN* locus may not have been previously identified as a telomere-related gene from GWAS studies because of insufficient power, the lack of a tagging SNP, differences in methods for measuring telomere lengths or, as this data suggest, epigenetic effects. These findings highlight the complexities of identifying genetic determinants of human telomere lengths. Caution is needed in the interpretation of genome-wide techniques such as linkage or association mapping in identifying telomere-related loci, as these results may be complicated by epigenetic effects.

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Figure 1.

Leu cocyte telomere lengths (TLs) do not cosegregate with heterozygous *PARN* (polyadenylation-specific ribonuclease deadenylation nuclease) mutations in a familial pulmonary fibrosis kindred. (A) Abridged pedigree of one kindred with familial pulmonary fibrosis and the cosegregating *PARN*c.529C>T (Gln177*) mutation. Individuals with pulmonary fibrosis are indicated by black filled symbols; individuals with blood leucocyte TLs <1st percentile, <5th percentile and <10th percentile are indicated by red, green and blue symbols, respectively. The arrow denotes the proband. Numbers in parentheses indicate individuals for whom no DNA sample is available. The presence or absence of the heterozygous *PARN* mutation is indicated by 'Mut' or 'WT', respectively. The age at the time of blood draw or death is indicated to the upper right of each symbol. (B) Genomic DNA telomere measured using a multiplexed quantitative PCR assay and expressed as age-adjusted (observed minus expected) TLs.³ (C) Genetic linkage analysis of kindred shown in A. Multipoint model-based analysis of genomic linkage identifies a peak on chromosome 16 with a maximal LOD score of 2.6 (p value = 2.5×10^{-3}) that does not encompass the *PARN*

gene (location indicated by the arrow). All affected individuals share a segment of 37.1 Mb identical-by-descent bounded by markers rs229007 and rs17281761.

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