Younger Age of Cancer Initiation Is Associated with Shorter Telomere Length in Li-Fraumeni Syndrome

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Abstract

Li-Fraumeni syndrome (LFS) is a cancer predisposition syndrome frequently associated with germ line *TP53* mutations. Unpredictable and disparate age of cancer onset is a major challenge in the management of LFS. Genetic modifiers, including the *MDM2*-SNP309 polymorphism, and genetic anticipation have been suggested as plausible explanations for young age of tumor onset, but the molecular mechanisms for these observations are unknown. We speculated that telomere attrition will increase genomic instability and cause earlier tumor onset in successive generations. We analyzed mean telomere length and *MDM2*-SNP309 polymorphism status in individuals from multiple LFS families and controls. A total of 45 peripheral blood lymphocyte samples were analyzed from 9 LFS families and 15 controls. High rate of *MDM2*-SNP309 was found in *TP53* carriers ($P = 0.0003$). In children, telomere length was shorter in carriers affected with cancer than in nonaffected carriers and wild-type controls ($P < 0.0001$). The same pattern was seen in adults ($P = 0.002$). Within each family, telomere length was shorter in children with cancer than in their nonaffected siblings and their noncarrier parents. Telomere attrition between children and adults was faster in carriers than in controls. Our results support the role of *MDM2*-SNP309 as a genetic modifier in LFS. The novel finding of accelerated telomere attrition in LFS suggests that telomere length could explain earlier age of onset in successive generations of the same family with identical *TP53/MDM2*-SNP309 genotypes. Furthermore, telomere shortening could predict genetic anticipation observed in LFS and may serve as the first rational biological marker for clinical monitoring of these patients. [Cancer Res 2007; 67(4):1415–8]

Introduction

Cancer is a genetic disease arising from a single cell. Most tumor types share abnormalities in common genetic pathways and mutations in distinctive genes. This fails to explain the differences in age of onset and severity of disease between affected patients with the same tumor. Li-Fraumeni syndrome (LFS) is a rare cancer predisposition syndrome frequently associated with germ line mutations in the *TP53* tumor suppressor gene (1). Furthermore, *TP53* plays a cardinal role in tumorigenesis and is mutated in at least 50% of sporadic tumors (2). LFS, therefore, can serve as an attractive model to assess different mechanisms that control tumor initiation in different individuals. In genetic diseases, variation in the age of onset is thought to be determined by two principle mechanisms: genetic modifiers (genes that modify or influence the severity of the already abnormal genetic pathway) and genetic anticipation (3). Anticipation is defined as higher incidence, earlier onset, or increased severity of a disease in successive generations. The molecular mechanisms governing anticipation are largely unknown except for generational expansion of trinucleotide repeats, which have been identified in a number of genetic diseases (4–6).

Genetic anticipation has been suggested to play an important role in LFS (7, 8). Recently, the *MDM2*-SNP309 polymorphism has been shown to be a plausible candidate for a genetic modifier in *TP53* mutated cancers (9–11) and in LFS. Murine double minute-2 (MDM2) is a key negative regulator of p53, which targets p53 toward proteasomal degradation. The SNP309 T>G variation, located in the first intron of *MDM2*, has been found to increase Sp1 transcription factor binding and, consequently, MDM2 expression levels. The 72Arg variant of the p53 protein has been shown to have a higher affinity toward MDM2 compared with the 72Pro variant, and, therefore, higher degradation of p53 is expected.

In addition, in dyskeratosis congenita, a bone marrow failure and premature aging syndrome, which is also associated with cancer predisposition, a striking association between telomere shortening and early onset and severity of disease has been found (12). The phenotypic hallmarks of dyskeratosis congenita are attributable to mutations in *TERC* and other genes in the telomerase complex, causing lack of telomerase activity. Lack of telomere maintenance is known to cause short dysfunctional telomeres that are associated not only with senescence but also with higher genomic instability and predisposition to cancer.

Based, in part, on these previous observations, we hypothesized that faster telomere attrition and the resulting shorter telomeres in offspring of *TP53* mutation carriers may be associated with the earlier onset of cancer in successive generations of LFS families.

Materials and Methods

To explore this hypothesis, we analyzed mean telomere length from peripheral blood lymphocytes of individuals from multiple LFS families with documented germ line *TP53* mutations (with and without cancer) and wild-type (WT) *TP53* controls. The study was approved by the Research Ethics Board at the Hospital for Sick Children in Toronto. Overall, 45 samples from 9 LFS families and 15 *TP53* WT germ line controls were collected after obtaining written informed consent (Fig. 1). Subjects were designated as either "children" (age at sampling < 18 years) or "adults" (age at sampling > 18 years) and subsequently categorized as affected (has/had cancer) germ line mutant *TP53* carriers, nonaffected mutant *TP53* carriers, and WT *TP53* carriers. Genomic DNA was extracted from peripheral blood leukocytes. *TP53* status was confirmed by sequencing exons 2 to 11 and intron-exon boundaries (13). The clinical

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and genetic data of our cohort are summarized in Fig. 1 and Table 1. *MDM2*-SNP309 was analyzed by sequencing the first intron of *MDM2* using primers 5′-GAGGTCTCCGCGGGAGTTC-3′ and 5′-TGCCCACT-GAACCGGC-3′ (14). In addition, *TP53* codon 72 polymorphism was analyzed by RFLP with the restriction enzyme *Bst*UI (New England Biolabs, Ipswich, MA) and the primers 5′-ATCTACAGTCCCCCTTGCCG-3′ and 5′-GCAACTGACCGTGCAAGTCA-3′ (1). Telomere length was assessed by the terminal restriction fragment assay using the Telo-TAGGG Telomere Length Assay kit (Roche, Mannheim, Germany) as previously described by our group (15). For statistical analysis of mean terminal restriction fragment between groups, Student's t test was applied. χ^2 was used for *MDM2*-SNP309 analysis.

Results and Discussion

The results of terminal restriction fragment *MDM2*-SNP309 and *TP53* codon 72 polymorphisms in our cohort are summarized in Table 2. We observed the non-WT *MDM2*-SNP309, which is associated with earlier onset of cancer in LFS patients, in 19 of 21 carriers in contrast to the expected 52% in the general population (ref. 9; $P = 0.0003$). This high *MDM2*-SNP309 frequency may be attributed to the inherent ascertainment bias in our population, which consists only of families with a proband who was affected as a child. The combination of non-WT *MDM2*-SNP309 and *TP53* codon 72 polymorphism, previously reported to be associated with the earliest onset of cancer in LFS and other cancers (14), was observed in six of eight affected children but only in one of four affected adults, suggesting that the carriers of the *MDM2*-SNP309/*TP53* codon 72 polymorphism combination will develop their first malignancy at a particularly earlier age. Although these data support the role of *MDM2*-SNP309 as a genetic modifier in LFS, they do not explain the difference in age of onset in individuals with the same genotype, specifically in members of the same family. Therefore, we assessed telomere length in our cohort. Telomere length was significantly shorter in affected than in nonaffected carriers and WT *TP53* controls (Fig. 2*B*). Moreover, telomere attrition over time, manifested by differences in telomere length between children and adults, was higher in mutant *TP53* carriers than in WT *TP53* controls (Fig. 2*B*). Figure 2*A* shows shorter telomeres in affected carriers versus noncarriers in two representative families. Telomeres were shorter in affected children than in their *TP53* WT relatives (parent/sibling) but not in their affected parent. These findings suggest accelerated telomere attrition as a novel and plausible biological mechanism to explain the observed anticipation phenotype in LFS.

Shorter and dysfunctional telomeres have been associated with progression from normal tissue through dysplasia to neoplasia in a variety of cancers (16, 17). Furthermore, individuals with cancer have shorter telomeres than normal age-matched controls (18). Our results support these findings from the unique context of a genetically based multigenerational cancer predisposition syndrome. It is not known why LFS patients have faster telomere attrition, but this feature has been found in other syndromes involving DNA repair abnormalities (19, 20). One can speculate that lack of *TP53* allows for cells, both somatic and germ line, with shorter dysfunctional telomeres, to escape senescence and proliferate. This would lead to shorter telomeres at birth in the next generation.

Combined with faster telomere attrition throughout life, this trend could possibly predict the risk and age of onset by determination of the threshold at which telomere length reaches a high probability of genomic instability leading to cancer (Fig. 2*C*). The dotted line in Fig. 2*C* represents telomere length, which serves as the threshold below which the risk of genomic instability and cancer initiation is high. Indeed, carriers born with short telomeres will reach the threshold and become affected early in life (all affected carriers had shorter telomeres than the threshold) whereas normal controls are not expected to reach this

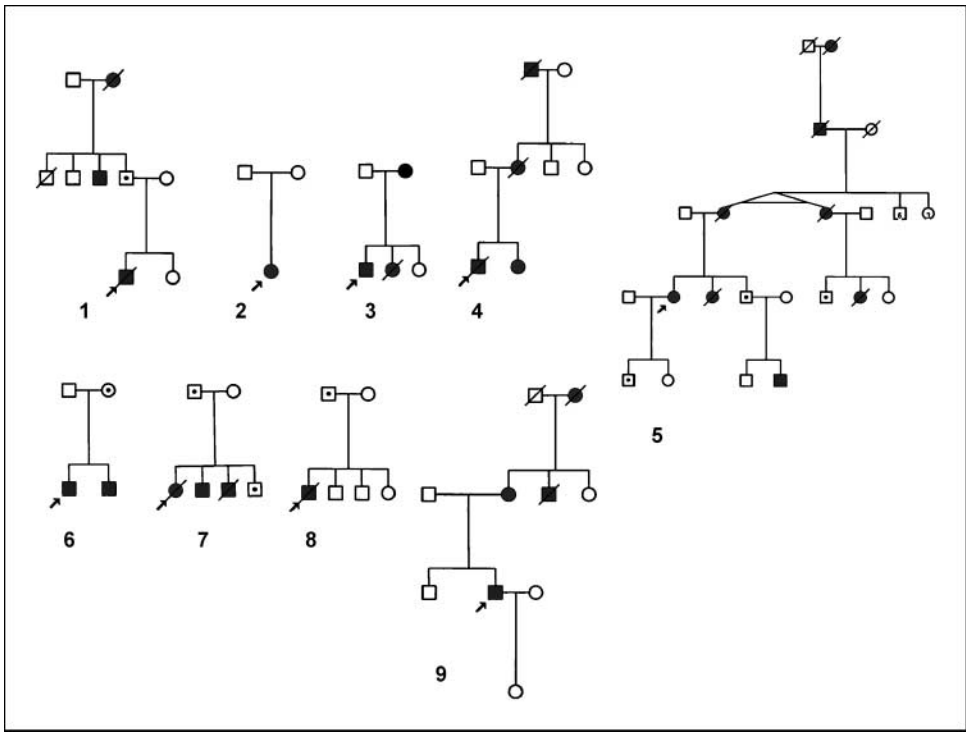

Figure 1. Pedigrees of study families with *TP53* mutations. *Open circles* and *squares,* normal females and males. *Black circles* and *squares,* affected females or males. A dot inside represents carrier status of a nonaffected individual. *Oblique line,* death. *Arrows,* proband in each family. The numbers of families correspond to those in Table 1.

Table 1. Clinical and genetic status of *TP53* mutation carriers

Family	Index	Age (y)	Status	*TP53* mutation	Tumor	Remarks
1	Proband	0.9	Affected	$Arg^{175}His$	CPC	Multiple tumors
	Father	30	Not affected	$Arg^{175}His$		
2	Proband	1.1	Affected	$His^{193}Pro$	CPC, ADCC	Multiple tumors No family history
3	Proband	6	Affected	$Ser^{273}Arg$	ADCC	Multiple tumors
	Mother	27	Affected	$Ser^{273}Arg$	MFH	
	Sister	7	Affected	$Ser^{273}Arg$	CPC	Multiple tumors
4	Proband	14	Affected	$Arg^{175}His$	Glioblastoma	
	Sister	21	Affected	$Arg^{175}His$	Breast cancer	
	Mother	26	Affected	$Arg^{175}His$	Breast cancer	Multiple tumors
5	Proband	25	Affected	IVS03-11 C>G	Breast cancer	
	Son	5	Not affected	IVS03-11 C>G		
6	Proband	3	Affected	$Arg^{158}His$	CPC	
	Brother	0	Affected	$Arg^{158}His$	Neuroblastoma	
	Mother	26	Not affected	$Arg^{158}His$		
7	Proband	2.5	Affected	$Arg^{248}Gln$	Medulloblastoma	
	Father	37	Not affected	$Arg^{248}Gln$		
	Brother	7	Not affected	$Arg^{248}Gln$		
	Brother	15	Affected	$Arg^{248}Gln$	Osteosarcoma	
8	Proband	2.7	Affected	12138 insC; pro72fs	RMS	Multiple tumors
	Father	39	Not affected	12138 insC; pro72fs		
9	Proband	2	Affected	$Pro^{152}Leu$	ADCC	
	Mother	47	Affected	$Pro^{152}Leu$	Breast cancer	Multiple tumors
	Maternal uncle	61	Affected	$Pro^{152}Leu$	MFH	

NOTE: Age of first cancer and/or blood sampling in *TP53* mutation carriers in the study. WT family members and controls are not included. The tumor type represents the first cancer in patients with multiple tumors.
Abbreviations: ADCC, adrenocortical carcinoma; CPC, choroid plexus carcinoma; MFH, malignant fibrous histiocytoma; RMS, rhabdomyosarcoma.

degree of telomere attrition during their lifetime. More importantly, nonaffected LFS carriers have longer telomeres at birth but faster rate of telomere attrition than normal individuals. Therefore, they are at risk of reaching the threshold later in life, depending on their initial telomere length at birth. This model is in agreement with the known lifetime risk of cancer in *TP53* mutation carriers, which approaches 100% in women and 80% in men. The practical aspect of our model is that given sufficient data, one would be able to predict the absolute risk and age of cancer initiation in LFS patients by using one or possibly two blood samples (allowing for initial telomere length and attrition rate). This information will be extremely important in planning the type and frequency of clinical surveillance screening tests for these patients.

Interestingly, one of the affected children did not fit this model. This patient (family 2, Table 1) exhibited much longer telomere length than the other affected children (11.1 kb versus a mean of 7.8 kb). This patient was later found to harbor a *de novo* mutation. That is, she has no family history of cancer and both her parents carry WT *TP53*. Therefore, it is tempting to speculate that the long telomeres actually predicted lack of *TP53* mutations in previous generations; therefore, this patient's results are compatible with our hypothesis. The exact reason for the early age of onset in this patient is probably related to other genetic modifiers and/or the target tissue because adrenocortical carcinoma is known to be commonly associated with *de novo* *TP53* mutations.

Our findings are in agreement with the importance of *MDM2*-SNP309 as a genetic modifier in LFS. Indeed, families carrying this SNP have a higher risk of having younger members affected. More importantly, our results provide a novel biological mechanism to

Table 2. Summary of study findings (n = 45)

Group	*N*	MDM2-SNP309 (non-WT)	SNP309 and *TP53* codon 72 polymorphisms	TRF, mean (SD)
Child				
Affected carrier	8	8 (100%)	6 (75%)	7.8 (0.46)
Nonaffected carrier	3	3 (100%)	1 (33%)	9.0 (0.26)
WT	15			9.1 (0.6)
Adult				
Affected carrier	5	4 (80%)	1 (20%)	6.8 (0.45)
Nonaffected carrier	4	3 (75%)	2 (50%)	7.7 (0.72)
WT	10			8.6 (0.9)

NOTE: Subjects were designated as either children (age at sampling, <18 years) or adults (age at sampling, >18 years) and subsequently categorized as affected (has/had cancer) germ line mutant *TP53* carriers, nonaffected mutant *TP53* carriers, and WT *TP53* carriers.
Abbreviation: TRF, terminal restriction fragment assay. Telomere length is measured in kilobase.

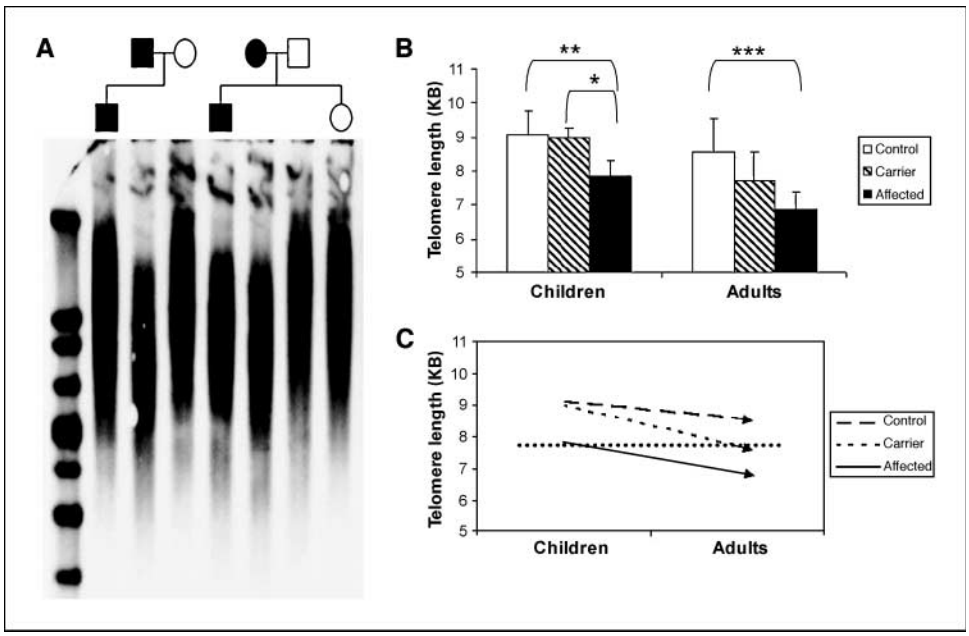

Figure 2. Telomere length in children and adults by clinical status. *A,* telomere restriction fragment measurement of two LFS families. In each family, the affected parent has shorter telomeres than their respective *TP53* WT spouse. The affected child has longer telomeres than the affected parent but shorter telomeres than their *TP53* WT sibling and even the *TP53* WT parent. *B,* mean telomere restriction fragment by study group. Telomeres were statistically shorter in affected subjects than in *TP53* WT controls. *C,* model showing trends of telomere shortening with age based on our findings (Fig. 2*B*). *Dotted line,* threshold below which shorter telomere length will yield higher genomic instability and cancer initiation process. Although mutant *TP53* carriers have accelerated telomere attrition compared with controls, only those who are born with short telomeres will be affected during childhood, whereas carriers with longer telomeres will be at risk later in life. The threshold could theoretically serve as a follow-up marker to guide initiation of clinical surveillance in LFS carriers. *, $P = 0.016$; **, $P < 0.0001$; ***, $P = 0.002$.

explain the earlier cancer onset in successive generations. Even within the same genotype, telomere shortening can predict the likelihood of being affected at a younger age.

Whereas the study is limited by a small sample size of a rare syndrome and should be interpreted accordingly, the significant difference between groups strengthens the validity of our observation and supports our hypothesis. Further studies to determine the role of telomere dysfunction in this phenomenon are ongoing.

Possible implications of this study include use of telomere length as a reliable marker for assessing the risk and the appropriate screening tests for LFS carriers. Moreover, we believe that our results highlight the role of telomere maintenance in cancer initiation, as well as progression, and should be expanded to other cancer predisposition syndromes.

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