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
and genetic data of our cohort are summarized in Fig. 1 and Table 1. 

**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. 2B). 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 carriers (Fig. 2B). Figure 2C 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. 2C). The dotted line in Fig. 2C 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
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 1. Clinical and genetic status of \(TP53\) mutation carriers

<table>
<thead>
<tr>
<th>Family</th>
<th>Index</th>
<th>Age (y)</th>
<th>Status</th>
<th>(TP53) mutation</th>
<th>Tumor</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Proband</td>
<td>0.9</td>
<td>Affected</td>
<td>Arg(^{175})His</td>
<td>CPC</td>
<td>Multiple tumors</td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td>30</td>
<td>Not affected</td>
<td>Arg(^{175})His</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Proband</td>
<td>1.1</td>
<td>Affected</td>
<td>His(^{195})Pro</td>
<td>CPC, ADCC</td>
<td>Multiple tumors</td>
</tr>
<tr>
<td></td>
<td>Mother</td>
<td>27</td>
<td>Affected</td>
<td>Ser(^{273})Arg</td>
<td>MFH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sister</td>
<td>7</td>
<td>Affected</td>
<td>Ser(^{273})Arg</td>
<td>CPC</td>
<td>Multiple tumors</td>
</tr>
<tr>
<td>3</td>
<td>Proband</td>
<td>6</td>
<td>Affected</td>
<td>Ser(^{273})Arg</td>
<td>ADCC</td>
<td>Multiple tumors</td>
</tr>
<tr>
<td></td>
<td>Mother</td>
<td>27</td>
<td>Affected</td>
<td>Ser(^{273})Arg</td>
<td>MFH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sister</td>
<td>7</td>
<td>Affected</td>
<td>Ser(^{273})Arg</td>
<td>CPC</td>
<td>Multiple tumors</td>
</tr>
<tr>
<td>4</td>
<td>Proband</td>
<td>14</td>
<td>Affected</td>
<td>Arg(^{175})His</td>
<td>Glioblastoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mother</td>
<td>26</td>
<td>Affected</td>
<td>Arg(^{175})His</td>
<td>Breast cancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Son</td>
<td>5</td>
<td>Not affected</td>
<td>Arg(^{175})His</td>
<td>CPC</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Proband</td>
<td>25</td>
<td>Affected</td>
<td>IVS03-11 C&gt;G</td>
<td>Breast cancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Son</td>
<td>5</td>
<td>Not affected</td>
<td>IVS03-11 C&gt;G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Proband</td>
<td>3</td>
<td>Affected</td>
<td>Arg(^{198})His</td>
<td>CPC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td>0</td>
<td>Affected</td>
<td>Arg(^{198})His</td>
<td>Neuroblastoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mother</td>
<td>26</td>
<td>Not affected</td>
<td>Arg(^{198})His</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Proband</td>
<td>2.5</td>
<td>Affected</td>
<td>Arg(^{248})Gln</td>
<td>Medulloblastoma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td>37</td>
<td>Not affected</td>
<td>Arg(^{248})Gln</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brother</td>
<td>7</td>
<td>Not affected</td>
<td>Arg(^{248})Gln</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brother</td>
<td>15</td>
<td>Affected</td>
<td>Arg(^{248})Gln</td>
<td>Osteosarcoma</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Proband</td>
<td>2.7</td>
<td>Affected</td>
<td>12138 insC; pro72fs</td>
<td>RMS</td>
<td>Multiple tumors</td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td>39</td>
<td>Not affected</td>
<td>12138 insC; pro72fs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Proband</td>
<td>2</td>
<td>Affected</td>
<td>Pro(^{152})Leu</td>
<td>ADCC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mother</td>
<td>47</td>
<td>Affected</td>
<td>Pro(^{152})Leu</td>
<td>Breast cancer</td>
<td>Multiple tumors</td>
</tr>
<tr>
<td></td>
<td>Maternal uncle</td>
<td>61</td>
<td>Affected</td>
<td>Pro(^{152})Leu</td>
<td>MFH</td>
<td></td>
</tr>
</tbody>
</table>

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.

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

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

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.
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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References

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