

# Risk Stratification in Cancer Predisposition Syndromes: Lessons Learned from Novel Molecular Developments in Li-Fraumeni Syndrome

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## Abstract

**Germ-line mutations in specific genes predispose family members to cancer. Prediction of the exact tumor type and timing of cancer initiation is fundamental to the development of management strategies for these individuals. Recent advances in our understanding of the general processes that control cancer initiation may enable us to tailor more precise risk stratification. This, in turn, will lead to more effective early detection strategies, which would result in more favorable clinical outcomes. In this review, we highlight the steps and methods used to reach this futuristic model.** [Cancer Res 2008;68(7):2053–7]

## Background

Clinical descriptions of familial cancer clusters, familial cancer predisposition syndromes (CPS), and the association of specific cancers occurring together with nonmalignant congenital anomalies had been variously described throughout the 20th century. However, Knudson's recognition of the statistical predictability of patterns of inheritance in hereditary retinoblastoma and Wilms' tumor in the early 1970s, together with the discovery of the retinoblastoma susceptibility tumor suppressor gene *Rb1* in 1986 (1), launched a new era in the field of cancer genetics. Several dozen CPS have been characterized clinically, and for over 30 of these, heritable germ-line mutations in tumor suppressor genes, oncogenes, or DNA stability genes have been identified, directly implicating these genes in the development of the cancer phenotype in these families (2). Nevertheless, some important fundamental questions remain unanswered: What are the processes that control the genetic penetrance of mutations in these genes? Can the genetic basis of the disease predict clinical severity and biological grade of the cancer? What is the risk of developing cancer, and the age of onset in an individual carrying the mutation? Resolving these questions will have major effects on the approach to management of such patients, including the development and timing of appropriate clinical screening tests and preventive interventions. Modifying cofactors, including environmental exposures (e.g., smoking, medications, nutrition, and infection), host-related factors (e.g., hormonal status, gender, and age), and other constitutional genetic and epigenetic events (e.g., modifier genes and methylation), complicate the risk estimates in these CPS. Poor understanding of the influence of these complicating factors has

confounded clinical risk estimation in many CPS. However, recent insights in one particularly intriguing CPS model have provided some clarity that should find relevance in many other similar disorders. In this review, we will focus on Li-Fraumeni syndrome (LFS), which is caused by germ-line mutations in the *TP53* gene and is a prototype CPS.

## Definition and the Problem

The constellation of tumors that was eventually coined "LFS" was originally reported by Li and Fraumeni in 1969. These tumors included early-onset soft tissue sarcomas and osteosarcomas, breast cancer, brain tumors, and leukemia. This definition has evolved to include a wider spectrum of tumors, including choroid plexus carcinoma, adrenocortical carcinoma, and other tumors. In 1990, germ-line mutations in *TP53* were first described by Malkin et al. (3) and Srivastava et al. (4) to be associated with the development of cancer in classic LFS families. Unlike many other CPS that exhibit a narrow tumor spectrum (e.g., *BRCA1* mutations cause almost exclusively breast and ovarian cancers), people harboring germ-line *TP53* mutations develop a wide variety of pathologically and teleologically distinct tumors presenting anytime from birth to late adulthood. Moreover, there seems to be a role for other genes in the *TP53* growth regulatory pathway involved in classic LFS (5). This further complicates the prediction and risk stratification of individuals with LFS. In the next paragraphs, we will elaborate on some of the approaches to refine the genetic basis of LFS and to use this information to generate rational clinical management and surveillance approaches to individual patients.

## Phenotype/Genotype

For each CPS in which a gene and its function are uncovered, a discussion about correlation between genotype and phenotype immediately follows. In many syndromes, including hereditary retinoblastoma, neurofibromatosis type 1, and hereditary breast/ovarian cancer syndrome, nonsense mutations or gene deletions that encode complete loss of protein function and expression are generally associated with higher penetrance and disease aggressiveness, whereas missense mutations may yield a milder phenotype (6–8). In LFS, nonsense, frameshift, and splice mutations, which are likely to result in loss of expression of the *TP53* protein or in a nonfunctional protein, seem to be associated with early onset of cancer especially in brain tumors (9). Missense mutations in the DNA-binding domain of the *TP53* gene are generally associated with higher incidence of breast and brain cancers, whereas adrenocortical tumors are the only group of tumors that are consistently associated with mutations outside the DNA-binding loops. This latter observation is especially intriguing

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because adrenocortical tumors are unique in that their mutations exhibit very low penetrance (<10% in contrast to >80% in families with no instances of adrenocortical tumors; ref. 10). Although these findings are important and may aid in management strategies for some LFS families, the diverse spectrum of tumors found in most LFS pedigrees does not lend themselves to obvious genotype-phenotype correlates. Moreover, in any particular family in whom all affected members harbor, by necessity, the same mutation, the variability in tumor type and ages of onset is often quite extreme, again precluding recognition of obvious *TP53* genotype-phenotype links. This led researchers to explore other potential genetic and epigenetic modifiers to account for the phenotypic variability.

## Host-Related and Environmental Factors

Environment plays an important role in carcinogenesis. Specific toxins, nutrients, carcinogens, and other factors contribute dramatically to specific cancer risk. The role of these factors has not been effectively addressed in LFS, primarily due to the relative rarity of the syndrome, its high penetrance, and the often lethal nature of the cancers that develop. Recently, Wu et al. (11) reported their observation that females with LFS are at higher risk than males of being affected. This supports previous work suggesting that whereas male germ-line mutant *TP53* gene carriers have a 75% lifetime risk of developing cancer, female carriers exhibit an ~100% lifetime risk. Although the high incidence of breast cancer must explain a significant proportion of this increased risk, the observation also does raise questions about the role of hormonal factors in cancer initiation in LFS. Although the role of nutritional status has not been explored in human LFS families, intriguing observations in *TP53*-deficient mice reveal that juvenile-onset calorie restriction delays tumor development in young *TP53*-null (-/-) mice through a *TP53*-dependent and insulin-like growth factor-I-related mechanism (12). A similar diet suppressed tumor development in heterozygous *TP53*-deficient [*TP53*(+/-)] mice even when initiated late in life (13). Further studies in human LFS families would be necessary to understand this phenomenon more clearly. Epigenetic inactivation of the retained wild-type *TP53* allele has been explored in an effort to explain unique tumor phenotypes in LFS. We have previously shown the selective expression and localization of SV40 poliovirus large T antigen, by both DNA sequence analysis and immunostaining, in choroid plexus carcinoma and renal cell carcinomas of two LFS patients with multiple tumors. The other tumor (rhabdomyosarcoma) in one of these patients did not harbor the viral particles but lost the normal *TP53* allele, suggesting that inactivation of *TP53* by viral proteins may contribute to tumor formation in carriers of mutant *TP53* (14). Finally, the role of therapeutic interventions in modifying the tumor phenotype is raised by reports indicating the development of metachronous tumors in the radiotherapy fields of a patient with a germ-line inactivating *TP53* mutation who had received radiation therapy for treatment of breast cancer (15). These findings are important in that they suggest that additional "hits" of the *TP53* gene itself or genetic modifiers of *TP53* function can result in dysfunction of the *TP53* cellular growth regulatory pathway and predispose to higher risk of cancer.

## Genetic Modifiers

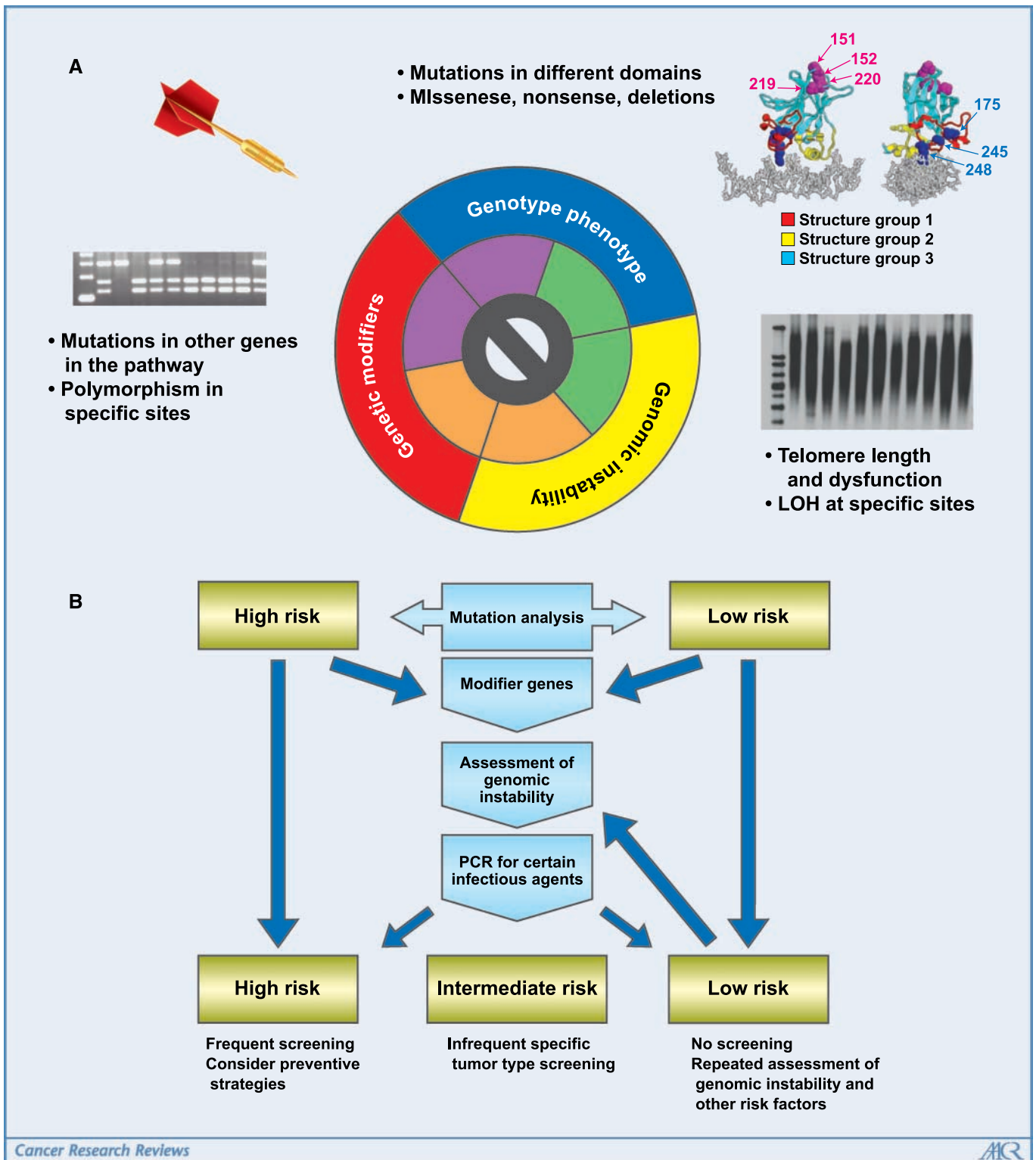
The search for genetic events that modify the phenotype of a CPS is being intensely pursued. Approaches include high-

throughput screening technologies to verify differences in somatic gene expression patterns in tumors from individuals harboring the same germ-line gene mutations. Screening for mutations or epigenetic events in other candidate genes, which have known roles in modifying the biological function of the index gene, is also an effective measure. Murine double minute 2 (*MDM2*) is a key negative regulator of *TP53*, targeting *TP53* protein toward proteasomal degradation. The SNP309 T>G variation, located in the first intron of *MDM2*, increases Sp1 transcription factor binding and, consequently, *MDM2* expression. The common *TP53* codon 72Arg polymorphism has been shown to have a higher affinity toward *MDM2* compared with the 72Pro isoform, leading to a higher degree of *TP53* degradation. Therefore, a cumulative effect is observed when both the *MDM2* SNP309 and the *TP53* 72Arg isoforms coexist (16–18). Numerous studies have been undertaken exploring the biological role of the *MDM2* SNP309 polymorphism in a spectrum of cancers. Whereas in some instances presence of the polymorphism seems to associate with increased risk for certain cancers (19), for others no such associations are observed, suggesting that any biological or phenotypic effect of the two variants may be very small (20, 21). Recently, the *MDM2* SNP309 polymorphism is a plausible candidate as a genetic modifier in *TP53*-mutated cancers and in LFS (16, 18).

However, this confluence of genetic modifiers does not fully explain observed differences in cancer phenotypes of individuals with the same *TP53* genotype, especially within the same family. The observation of genetic anticipation in LFS (22) suggests a role of additional hits or higher mutator phenotypes with successive generations within these families.

## Genetic Anticipation

The essence of genetic anticipation is the progressive severity and/or earlier age of onset of illness with successive generations, leading ultimately to extinction of the lineage. In the setting of cancer, genetic anticipation is characterized by an earlier age of disease onset, increased severity, and a greater proportion of affected individuals in succeeding generations. The molecular mechanisms governing anticipation are largely unknown; the few described are not generally associated with cancer predisposition (23). Intriguingly, in dyskeratosis congenita (DC), a bone marrow failure and premature aging syndrome, an association between telomere shortening and early onset and severity of disease has been found (24). Strikingly, dyskeratosis congenita is also associated with cancer predisposition. Variability in phenotypic expression of DC is accounted for by mutations in genes encoding the telomerase complex. The autosomal dominant form of the disease is attributable to mutations in *TERC*, the RNA component of telomerase. Heterozygous mutations in the reverse transcriptase component of telomerase often lead to incomplete penetrance and a diverse clinical presentation. The autosomal recessive form of DC is genetically heterogeneous, with one subtype being attributed to mutations in the *NOP10* gene that encodes a protein associated with maturation of rRNA and the telomerase complex (25). Ultimately, mutations in these genes all lead to lack of telomerase activity, telomere attrition, and cellular senescence. This lack of telomere maintenance is known to cause short dysfunctional telomeres, which is associated not only with senescence but also with higher genomic instability and predisposition to cancer.



**Figure 1.** Models of an approach to CPS. *A*, the cancer predisposition dartboard. Several genetic risk factors contribute to the chance of being affected. Each combination will lead to increased risk. Combination of several risk factors will likely lead to 100% penetrance. New assays will enable us to determine the exact risk and manage the individual accordingly. The structure group representation is adapted from M. Olivier et al. (9). *B*, management of an individual with family history of cancer. After relevant gene mutation screening and analysis is completed, genotype-phenotype correlations will determine initial risk groups. Then, a battery of relevant assays to determine genetic modifiers, genomic instability, and other testing will determine final risk stratification. Individuals with low risk will only be assessed for degree of genomic instability and certain pathogens every several years. High-risk individuals will be assigned to frequent multimodality screening and possible tumor-preventive procedures or therapies.

## Telomere Dysfunction and Genomic Instability in LFS

As the guardian of the genome, TP53 protects cells from many types of DNA damage. Activation of TP53 results in cell cycle arrest, which allows for other partners of the DNA damage machinery to repair the genomic damage. In the setting of irreversible damage, TP53 activation leads to apoptosis or senescence. Therefore, TP53-deficient cells will accumulate genetic damage and dysfunctional telomeres leading to genomic instability. Although the spectrum of tumors that develop in TP53-deficient mice does not specifically resemble the human LFS counterpart, telomerase-deficient/TP53-mutant mice develop epithelial cancers in successive generations through telomere dysfunction and nonreciprocal chromosomal translocations, which closely mimic the multigenerational genetic anticipation observed in the LFS phenotype (26). Other models of accelerated telomere attrition include Fanconi anemia and ataxia-telangiectasia in which alterations in other genes of the DNA damage response machinery occur (27). These molecular and biological observations suggest that perhaps the combined effect of accumulated additional genetic "mistakes" that are ineffectively repaired in the presence of mutant TP53 and shorter telomeres over time in LFS individuals will lead to greater genomic instability in successive generations and earlier age of cancer onset. We recently reported observations to support this hypothesis by showing that LFS individuals exhibit accelerated telomere attrition compared to controls. Furthermore, earlier onset of cancer in successive generations of LFS families was associated with presence of shorter telomeres in peripheral blood lymphocytes (17). Similar observations were recently reported by Trkova et al. (28). These findings led us to suggest the following alternative model for the management and surveillance of patients with LFS.

### Plausible Model for Management of LFS

On recognition of an individual's potential to harbor a germ-line *TP53* mutation based on clinical criteria (family or personal cancer history), *TP53* mutation analysis is undertaken. After determining the specific genotype, including the *TP53* codon 72 and *MDM2* SNP309 polymorphism status, the individual can be categorized to belong to high- or low-risk groups for presymptomatic clinical surveillance. To a certain extent, specific *TP53* genotype-phenotype correlates may be applied, but these will be enhanced by further gene evaluations (9). Telomere length or measurement of telomere dysfunction will assess whether the patient lies under a defined

threshold to develop cancer (Fig. 1A) and over what period this particular risk is anticipated to become apparent (17). As additional markers of genomic instability are developed, it may be possible to further refine this risk. People at immediate risk based on very rapid telomere attrition or significant telomere dysfunction would be screened more frequently for specific types of tumor, whereas for the lower-risk individuals a less stringent surveillance follow-up would be applied (Fig. 1B). The importance of such an approach would be to identify cancers earlier in the hopes of enhancing survival advantages for those at exceedingly high risk while limiting exposure to potentially invasive surveillance modalities for those *TP53*-mutant gene carriers deemed at particularly low risk or at risk of organ-specific tumors that may be detected by specific surveillance tests. Because few individual centers follow sufficient LFS families to evaluate the efficacy of such an approach in a timely manner, the feasibility of this surveillance model will require evaluation in a multi-institutional prospective fashion.

### Final Remarks

In this review, we summarized the common biological and molecular mechanisms that determine the phenotype in CPS. We used the paradigm of LFS to highlight the role of genomic instability in predicting the development of imminent cancer. In addition, we point to the potential power of using a complex of diagnostic molecular markers to refine genetic risk evaluation in the setting of a common *TP53* germ-line mutation. Although accelerated telomere attrition is one such modifying marker, measurement of telomere length per se is a relatively crude way to assess such a complex process. Moreover, mean telomere length is less biologically relevant than other methods to assess telomere dysfunction because in each cell only the shortest and dysfunctional telomeres determine its fate. Nevertheless, as techniques continue to evolve to more accurately quantify genomic instability, we believe that this concept may have clinical implications in the near future.

Importantly, although we focused on LFS, we also would predict that this concept can be expanded to other CPS in which DNA repair and genomic instability play a major role.

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