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Li-Fraumeni Syndrome

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Abstract

Li-Fraumeni syndrome (LFS) is a classic cancer predisposition disorder that is commonly associated with germline mutations of the *p53* tumor suppressor gene. Examination of the wide spectrum of adult-onset and childhood cancers and the distribution of *p53* mutations has led to a greater understanding of cancer genotype-phenotype correlations. However, the complex LFS phenotype is not readily explained by the simple identification of germline *p53* mutations in affected individuals. Recent work has identified genetic events that modify the LFS phenotype. These include intragenic polymorphisms, mutations/polymorphisms of genes in the p53 regulatory pathway, as well as more global events such as aberrant copy number variation and telomere attrition. These genetic events may, in part, explain the breadth of tumor histiotypes within and across LFS families, the apparent accelerated age of onset within families, and the range of clinical outcomes among affected family members. This review will examine the clinical and genetic definitions of LFS and offer insight into how lessons learned from the study of this rare disorder may inform similar questions in other familial cancer syndromes.

Keywords: Li-Fraumeni syndrome, cancer predisposition, germline p53 mutations

Clinical Definitions of Li-Fraumeni Syndrome

In 1969, a remarkable cancer predisposition syndrome was reported by Li and Fraumeni. Using a classic epidemiological approach, they retrospectively evaluated 280 medical charts and 418 death certificates of children diagnosed with rhabdomyosarcoma in the United States from 1960 to 1964.[1,2] Five families were identified in whom a second child had developed a soft tissue sarcoma. In addition, a high frequency of diverse cancer types was observed among first- and second-degree adult relatives along one ancestral line of each proband with cancer rates considerably in excess of those expected by chance alone. In addition to soft tissue sarcomas and premenopausal breast cancers, carcinomas of the lung, skin, pancreas or adrenal cortex, leukemia, and various brain tumors were also observed. Multiple metachronous primary neoplasms were also observed in several family members. Li and Fraumeni suggested that the occurrence of diverse neoplasms in these families might represent a counterpart of the tendency for a single individual to develop multiple primary tumors and that these families represented a previously undescribed familial cancer syndrome, with transmission suggestive of an autosomal dominant gene.

Based on prospective analysis of 24 families, the "classic" Li-Fraumeni syndrome (LFS) (OMIM #151623) pedigree was defined as a proband with sarcoma diagnosed under age 45 years, who has a first-degree relative with any cancer under 45 years, plus another first- or second-degree relative with either any cancer under 45 years or a sarcoma at any age.[3] An example of a "classic" LFS family is shown in Figure 1. To date, more than 500 LFS families have been reported and are either cited in the database of the International Association for Research on Cancer (IARC), in Lyon, France (http://iarc.p53/fr), or through isolated reports. As more families have been ascertained, the list of possible or probable component tumors has been expanded to include choroid plexus carcinoma, gastric cancer, lymphoma, melanoma, germ cell tumor, Wilms tumor, and colorectal cancer.[4] While these other cancers are only infrequently reported, compared with the "classic component tumors" noted above, they are particularly noteworthy in that they occur at strikingly younger ages than would be predicted from their sporadic occurrence in the general population. Families that do not conform to the criteria of classic LFS have been termed "LFS-like (LFS-L)."[5] These families were initially defined on the basis of a proband with any childhood cancer or sarcoma, brain tumor, or adrenocortical carcinoma diagnosed under 45 years of age with one first- or second-degree relative with a typical LFS cancer diagnosed at any age, plus a first- or second-degree relative in the same parental lineage with any cancer diagnosed under the age of 60 years. Chompret *et al*.[6] refined this definition further as the following: 1) a proband with a characteristic LFS tumor (sarcoma, brain tumor, breast cancer, adrenocortical carcinoma) before 36 years who has at least one first- or second-degree relative with a characteristic LFS tumor (other than breast cancer, if the proband has breast cancer) before 46 years; 2) a proband with multiple tumors, 2 of which represent characteristic LFS tumors and the first of which occurred before 36 years; or 3) a proband with adrenocortical carcinoma whatever the

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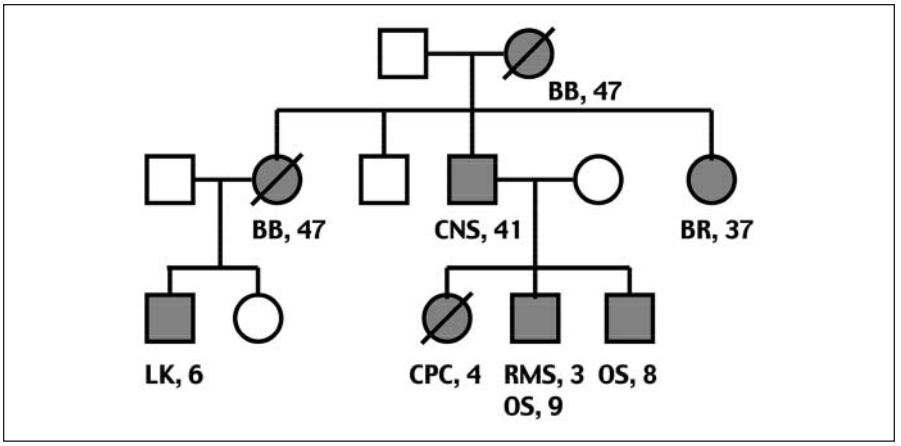

Figure 1. Pedigree of a family with Li-Fraumeni syndrome. Filled circles/squares represent affected members; slashes represent deceased family members. Numbers represent age at diagnosis. BB = bilateral breast cancer; CNS = brain tumor; BR = unilateral breast cancer; LK = leukemia; CPC = choroid plexus carcinoma; RMS = rhabdomyosarcoma; OS = osteosarcoma.

age of onset or family history. Recently, this definition has again been modified, reflecting more comprehensive genotyping studies (see below) of kindred. The "Revised Chompret" criteria[7] increase the age of tumor onset and focus on unique subsets of pediatric cancers for which genetic testing in the absence of a family history should be considered. In addition to the wide spectrum of tumor types observed in LFS, Hisada *et al*. showed that gene carriers are at significant risk of developing multiple synchronousormetachronousnontherapy-induced neoplasms.[8] In a retrospective study of 200 cancer-affected carriers of TP53 germline mutations, 15% developed a second cancer, 4% a third cancer, and 2% a fourth cancer, with survivors of childhood malignancies being those with the highest risk of developing additional malignancies.[8] The overall relative risk of occurrence of a second cancer was 5.3 (95% confidence interval [CI] = 2.8-7.8), with a cumulative probability of second cancer occurrence of 57%. Importantly, however, primarily because of the lack of prospective studies within the same treatment area, it is not clear whether risk of second cancers is similarly increased in the radiation field of p53 mutation carriers treated for a primary tumor nor whether the outcome of these patients (or LFS patients in general) is significantly different than non-LFS patients who are treated in a similar manner for the same sporadic tumor.

Cancer Risk Patterns in LFS Families

Even prior to identification of the gene associated with the majority of LFS cases, epidemiological studies defined remarkable cancer risk patterns within families. In one hospital-based analysis, the lifetime cancer risk of gene mutation carriers was estimated to be 73% in males and nearly 100% in females, with the high risk of breast cancer accounting for the difference. The specific risk for males is 19%, 27%, and 54% before the age of 15 years, 16 to 45 years, and >45 years, respectively. The risk for females is 12%, 82%, and 100% before the age of 15 years, 16 to 45 years, and >45 years, respectively.[9] A study by Hwang *et al*.[10] described the cancer risk in kindred ascertained on the basis of childhood soft tissue sarcomas. Cancer risk was determined for gene mutation carriers and noncarriers who had been followed for greater than 20 years. Among the carriers, 12%, 35%, 52%, and 80% developed cancer by ages 20, 30, 40, and 50 years, respectively. The most common cancers were breast cancer and soft tissue sarcomas. The 3,201 noncarriers had a cumulative risk of 0.7%, 1.0%, 2.2%, and 5.1% for the same ages, respectively, which is almost identical to that of the general population. While the number of carriers was similar in males and females, the cancer risks were not. The observed cancer risk was significantly higher in female carriers than males and, in contrast to the previous study presented, was not due to the incidence of breast cancer. At every age analyzed, females had a significantly higher incidence of cancer ($P < 0.001$). The specific cumulative risks for female carriers were found to be 18%, 49%, 77%, and 93% by ages 20, 30, 40, and 50 years, respectively, compared with cumulative risks of 10%, 21%, 33%, and 68% in male carriers at the same ages. Even after excluding sex-specific cancers (breast, ovarian, and prostate cancer), a higher female cancer risk was observed, including a higher risk for brain and lung cancer. Notwithstanding the intense interest in this unusual and highly penetrant syndrome, it was 20 years from its first description before the etiology of LFS was discovered.

p53 and LFS

In 1990, a candidate gene approach was taken to determine the underlying genetic lesion in LFS.[11] Based on earlier observations that somatic mutations of the *p53* tumor suppressor gene were observed in greater than 50% of sporadic human cancers[12] and that *p53* transgenic mice carrying mutant *p53* alleles developed a wide spectrum of malignancies,[13] p53 was examined in the constitutional DNA of LFS kindreds. While heterozygous point mutations were initially detected in 5 of 5 families, numerous subsequent studies have since shown that only 60% to 80% of "classic" LFS families harbor detectable germline *p53* mutations,[14,15] while the majority of LFS-like families do not harbor detectable *p53* mutations in the coding regions of the gene.[14,16] Studies in the United States and in Europe have suggested that germline *p53* mutations occur at the rate of about 1:5,000 individuals.[17] The lack of 100% concordance between

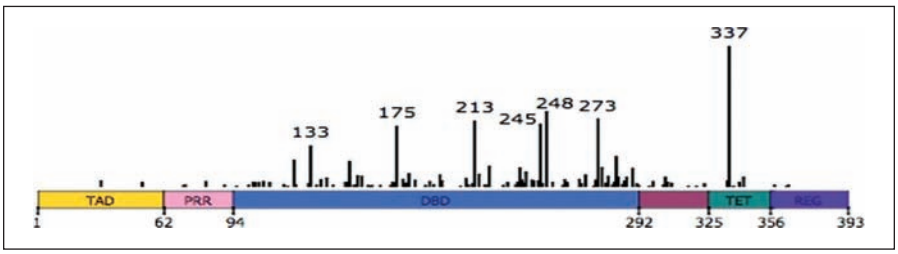

Figure 2. Relative frequency of germline mutations in *p53* by codon adjacent to the primary structure of the p53 protein. TAD = transactivation domain; PRR = proline-rich region; DBD = DNA binding domain; TET = tetramerization domain; REG = regulatory domain. Adapted from the International Association for Research on Cancer (IARC) database (Revision 14, November 2009).

p53 mutations and the classic phenotype may be explained in several ways, including posttranslational p53 alterations, complete *p53* deletion, the effects of modifier genes, or alterations of other genes influencing the phenotype generated by the presence of specific germline alterations. Recently, lesions within introns or the regulatory regions of the gene have been identified, although their functional significance is unclear. The spectrum of mutations detected in the germline reflects those found in sporadic tumors; the majority occurs within the DNA binding domain of the gene, primarily confined to highly conserved regions (Fig. 2). However, it is important to note that of the germline mutations found to date, few are located outside the coding regions of exons 5 to 8.[18] The most common mutations found in both sporadic tumors and in the germline are in codons 175, 245, 248, 273, and 282, although their order of frequency varies between the 2 groups[19] (http://www-p53.iarc.fr/index.html).

Functional analysis of the germline *p53* mutations has been carried out to establish the significance of these mutations and structural features of the corresponding p53 proteins. *In vitro* analysis of germline p53 alterations reveals that not all are associated with inhibition of growth arrest, apoptosis, transcriptional activation, or increased cancer risk. In fact, it is believed that in humans, the limited organ or target cell specificity of *p53* mutations may be due to varying genetic backgrounds, acquisition of subsequent gene alterations in target tissues, or the influence of epigenetic or environmental factors. Importantly, these studies determined that certain *p53* mutations might change the amino acid sequence in a conserved domain yet are not associated with an increased risk of cancer.[20] As well, these studies were able to explain the functional significance of heterozygous germline mutations, such as those at codon 245, by showing the transdominant effect of some mutant *p53* alleles on wild-type p53 DNA binding and T-antigen binding.[21] Inactivating mutations of the *p53* gene and disruptions of the p53 protein are observed in some fraction of virtually every sporadically occurring malignancy. In fact, as of November 2010, the IARC *TP53* Mutation Database identified the existence of 26,597 somatic mutations reported in 1,769 original publications, 535 germline mutations reported in 2,197 publications, as well as functional information on 2,314 mutant proteins (http://www-p53.iarc.fr/index.html).

The Role of Other Genes in LFS

The absence of detectable germline *p53* mutations in some LFS families has suggested the involvement of other genes, but this hypothesis remains controversial. Numerous genes involved in P53 pathway, apoptosis, or cell cycle control such as *p63*,[22] *BCL10*,[23] *BAX*,[24] *CDKN2A*,[25,26] *PTEN*,[25,27] and *CHEK1*[28,29] have been considered as candidate genes for LFS, but all these studies have provided negative results. Germline mutations of *CHEK2*, encoding a kinase able to phosphorylate Cdc25c and P53, were initially reported in 1 LFS family and 2 families suggestive of LFS,[28] but one alleged mutation, 1422delT, was subsequently shown to be on a duplicated exon.[30] The 2 other reported mutations, Ile157Thr and 1100delC, found in a total of 4 families suggestive of LFS[28,29] were subsequently shown to be polymorphisms, whose allele frequency has been, respectively, estimated to be 0.12% to 1.4% and 2.4% in European populations and which confer an increased risk for breast, prostate, and thyroid cancer.[31-33] These data argue against any major involvement of CHEK2 in LFS. A linkage to chromosome 1q23 was reported in a LFS family,[34] but the implication of a second locus in LFS remains to be confirmed.

Modifier Genes in LFS

The variability in age of onset and type of cancer among LFS families suggests effects of modifier genes on the underlying mutant *p53* genotype. Analysis of mutant genotype-phenotype correlations reveals intriguing observations. Nonsense, frameshift, and splice mutations yield truncated or nonfunctional proteins that are commonly associated with early-onset cancers, particularly brain tumors.[15] Missense mutations in the *p53* DNA binding domain are frequently observed in the setting of breast and brain tumors, while adrenocortical cancers are the only group that is associated with mutations in the non-DNA binding loops. Age of onset modifiers has also now been established. Recently, the *MDM2*-SNP309 polymorphism has been shown to be a plausible candidate as a genetic modifier in *p53* mutated cancers and in LFS.[35,36] Murine double minute 2 (MDM2) is a key negative regulator of P53, 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 levels. The mean age of tumor onset in MDM2 SNP309 carriers is significantly less than that observed in patients homozygous for the T allele.[37] The common *p53* codon 72Arg polymorphism has been

shown to have a higher affinity toward MDM2 compared with the 72Pro isoform, leading to a higher degree of P53 degradation. The mean age of tumor onset in *p53* codon 72Arg allele carriers is significantly less than that of Pro:Pro variant carriers.[37] Furthermore, a cumulative effect is observed when both the *MDM2* SNP309 and the *p53* 72Arg isoform coexist,[35,36] suggesting that the early onset conferred by the *MDM2* SNP309 polymorphism is amplified by the presence of the *p53* codon 72Arg polymorphism.[38] However, a recent analysis of 19 extended LFS pedigrees showed that while the *TP53* germline mutation and its interaction with gender were strongly associated with familial cancer incidence, the association between *MDM2* SNP309 and increased cancer risk was modest.[39] In fact, the interaction between *MDM2* SNP309 and *TP53* mutation was not statistically significant. The SNP309 G alleles were associated with accelerated tumor formation in both carriers and noncarriers of germline *TP53* mutations, suggesting that this was not an LFS-specific modifier effect. However, this confluence of genetic modifiers does not fully explain observed differences in cancer phenotypes of individuals with the same *p53* genotype, especially within the same family. The observation of genetic anticipation in LFS[40] suggests a role of additional "hits" or higher mutator phenotypes with successive generations within these families. The earlier age of onset of cancers with subsequent generations in mutant *p53* LFS families suggests genetic anticipation. This observation can be explained by accelerated telomere attrition from generation to generation.[41,42] In children in both these studies, telomere length was shorter in p53 mutation carriers affected with cancer than in nonaffected carriers and wild-type controls. The same pattern was seen in adults. 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. Thus, telomere shortening could predict genetic anticipation observed in LFS and has been suggested as a rational biological marker for clinical monitoring of these patients.

Three *p53* polymorphisms are in linkage disequilibrium within a 312-bp stretch of genomic DNA, including a SNP in intron 2 (PIN2), a 16-bp duplication in intron 3 (PIN3), and the SNP at codon 72 (PEX4). In a population of LFS patients harboring a unique mutation at codon 337, PIN3 has a significant effect on age of onset, with carriers of 1 minor allele (16-bp duplication) developing their first cancer, on average, 17.1 years later than carriers of 2 major (nonduplicated) alleles.[43] Haplotype analysis combining the 3 polymorphisms demonstrated that PEX4 (codon 72) has no independent effect. Further studies are needed to determine whether the effect of PIN3 is particularly important in R337H carriers or if it is a general effect in all germline *p53* mutation carriers. Recently, using both high-resolution Affymetrix GeneChip 250K Nsp and Sty SNP arrays (Santa Clara, CA) and Affymetrix 6.0GW SNP/CNV arrays, it has been demonstrated that the *p53* mutation carriers harbor a higher frequency of DNA copy number variable regions in their genome.[44,45] In addition to the excess CNV frequency that could reflect the underlying genomic instability conferred by a primary germline p53 mutation, other observations suggest a significant role of CNVs in LFS. The CNVs in p53 mutation carriers themselves frequently encompass cancer genes; CNVs from one parent are commonly inherited along with a germline p53 mutation from the other parent, with the confluence of these 2 genetic events conferring a particularly aggressive phenotype in the offspring; and CNVs identified in the germline appear to commonly progress in somatic (transformed) cells in the tumor, suggesting that they are in fact dynamic genomic elements. The increased copy number variable regions may be a reflection of the underlying genomic instability conferred by the *p53* mutations in these individuals. The precise mechanism of CNV formation is not entirely understood, although nonallelic homologous recombination (NAHR) and nonhomologous end joining (NHEJ) are thought to be involved.[46] Small intragenic deletions in the *TP53* coding region have been rarely observed in LFS patients. Extending the CNV model further, Shlien *et al*. demonstrated that while this is generally the case, large deletions that encompass the entire gene as well as up to 2 Mb upstream and downstream confer a distinctive phenotype that does not appear to confer an increased cancer risk. Rather, these individuals have a complex phenotype of congenital anomalies and global developmental delay. Remarkably, the monoallelic expression of an intact p53 gene is sufficient in these individuals to "protect" against the cancer phenotype, whereas deletion of the gene itself (or a substantial portion of it) confers a high cancer risk as would be observed with the more common point mutations. It is postulated from this work that the transforming events conferred by aberrant p53 expression in LFS result from qualitative abnormalities of the p53-expressed project rather than quantitative decrease in baseline expression.[47] Thus, while germline *p53* mutations establish the baseline risk of tumor development in LFS, a complex interplay of modifying genetic cofactors likely defines the specific phenotypes of individual patients.

The Unique Brazilian LFS-p53 Codon 337 Mutation Phenotype

In Brazil, a specific germline mutation at codon R337H (c.1010 G>A, genomic nucleotide number 17588) in exon 10, encoding the oligomerization domain of p53, was first identified in children with adrenocortical carcinoma (ADC) in families with no reported history of cancer.[48,49] Based on the analysis of 4 hypervariable loci on the short arm of chromosome 17, it had been concluded that the mutation might have arisen independently in different patients perhaps because of an

environmental mutagen. However, using 2 *p53* intragenic hypervariable loci in a larger group of cases and controls, Pinto *et al*.[50] suggested that a founder effect was statistically probable. The allele frequency of R337H in the population of Southeast and Southern Brazil is about 15 times higher than any other single *p53* mutation associated with LFS.[51] With pH in the low to normal physiological range (up to 7.5), the mutant protein forms normal oligomers and retains its suppressor function. However, at high physiological pH, the histidine replacing arginine at codon 337 becomes deprotonated and is unable to donate a hydrogen bond critical for protein dimerization. This prevents p53 from assembling into a functional transcription factor. This unique biochemical feature might contribute to the particular features of R337H families, which often show incomplete penetrance and heterogeneous tumor patterns.[52]

Analysis of tumor patterns in R337H carriers and their families revealed all the common features of LFS/LFL, clearly establishing that this mutant predisposes to a wide spectrum of multiple cancers. In R337H carriers, the penetrance at age 30 years is less than 20% (compared to about 50% in "classic" LFS). However, the penetrance over a lifetime is about 90%, similar to "classic" LFS. Interestingly, the mutation appears to be particularly prevalent in Southeast and Southern Brazilian populations, where the allele frequency is suggested to be about 0.0015.[53]

The high prevalence of a rare mutation raised the question of a possible founder effect among Brazilian family carriers of the same alteration. Using a dense panel of SNPs encompassing the whole *p53* gene revealed the presence of a rare haplotype, with a probability that the mutation arose independently on this haplotype of less than 10^{-8},[54] establishing the existence of a founder effect. Given the high population density in these areas, mutations might be present in several hundred thousand subjects and could explain the high frequency of many cancers including colorectal cancer and the greater than 15-fold increase in childhood adrenocortical cancer in the Brazilian population.

Functional Models of Germline p53 Mutations

Since the initial identification of germline *p53* mutations in LFS, *in vitro* transfection of tumor cell lines with plasmids carrying germline *p53* mutations indicated that mutations compromise the ability of P53 to inhibit the growth of malignant cells *in vitro* and to transactivate reporter plasmids containing P53 binding sites.[55,56] To systematically evaluate the effects of *p53* mutations on the transcriptional activity of the protein, which underlies its ability to control cell cycle, apoptosis, and DNA repair, functional assays in yeast were developed.[57,58] The FASAY (functional analysis of separated allele in yeast) is now widely used to detect germline or somatic *p53* mutations. The assay is based on the cotransformation of a reporter yeast strain with PCR-amplified *p53* cDNA (between codons 53 and 364), derived from patients' lymphocytes, a gapped expression vector linearized between codons 67 and 346, and the cloning of the cDNAs by homologous recombination. The activation by wild-type p53 of the reporter system, containing the ADE2 open reading frame cloned downstream of p53 binding sites, changes the color of the yeast colonies (red to white). Heterozygote inactivating mutations yield about 50% red colonies. FASAY analysis of lymphocytes from LFS patients harboring germline *p53* mutations has demonstrated that all the missense mutations result in a loss of function. Missense mutations confer oncogenic properties *in vitro*, and this gain of function could be explained either by the ability of mutant p53 to transactivate inappropriate target genes involved in cell cycle[59] or by a transdominant negative effect, whereby missense mutants interfere with the DNA binding of the wild-type protein.[60] Therefore, although the common biological effect of germline *p53* mutations is a loss of function, it is likely that some missense mutations may have a dual effect.

Furthermore, analysis of LFS fibroblasts or lymphocytes harboring *p53* heterozygous mutation has revealed striking differences with normal cells, in terms of chromosomal stability, apoptotic response to ionizing radiations, G2 arrest after DNA damage, and gene expression profiles.[61-64] This strongly suggests that heterozygous *p53* mutations have a biological effect, contributing to genetic instability and therefore facilitating the appearance of a second hit.

Mouse Models of LFS

In 1992, homozygous knockout mice with a germline *p53* deletion were shown to be developmentally normal but highly susceptible to early tumors.[65] Subsequent p53-null mice with different deletions of the p53 allele showed similar tumorigenic phenotypes.[66-68] The majority of p53-null mice developed T and B cell lymphomas within 6 months of age.[65-69] A closer genetic model for LFS, however, was the p53 mutant heterozygous mice because affected LFS individuals are invariably heterozygous rather than homozygous for mutant p53. Genetically, the p53-null heterozygous mice are a model for a significant fraction of LFS germline mutations that are functionally null for p53.[16]

Given the genetic similarity between the p53-null heterozygous mice and the subset of LFS lineages with null mutations, it is of interest to explore phenotypic similarities. Approximately 50% of heterozygous p53-null mice succumb to tumors by 18 months of age.[69,70] On a lifespan basis of 36 months for C57BL/6 mice, this is not too dissimilar from the 50% incidence that has been observed for affected members of LFS families, with a 50% incidence of cancers by age 30 years. With respect to the tumor spectrum, like the LFS families, the *p53*-null heterozygous mice exhibit high numbers of osteosarcomas and soft

tissue sarcomas but also display high numbers of B cell lymphoma/leukemia, a tumor type only weakly associated with LFS. In contrast to the high frequency of breast cancers in LFS, there were few mammary carcinomas in the *p53*-null heterozygous mice, although this may be largely because of the predominantly C57BL/6 background of the study population,[69] as C57BL/6 mice are highly resistant to mammary carcinomas.[71] When the *p53*-null allele was backcrossed into a mammary tumor–susceptible Balb/c background, 55% of the female heterozygotes developed mammary adenocarcinomas.[71] Thus, strain-associated modifier genes may greatly influence the types of tumors arising in the *p53*-deficient mice. It also suggests that appropriate manipulation of the strain background in the p53 heterozygous mice could be exploited to generate a mouse with similar tumor spectra as LFS patients.

In general, tumor suppressor genes such as *p53* are considered to be recessive. In those familial syndromes resulting from inheritance of a single defective tumor suppressor allele, loss or mutation of the second allele is often observed in the tumors that arise in these syndromes. P53 appears to be an exception to this rule. While mutation and loss of both *p53* alleles occur quite frequently in sporadically arising cancers, the tumors arising in LFS patients often retain a structurally intact wild-type *p53* allele.[72] Interestingly, two thirds of the tumors in those patients inheriting missense mutations in the central DNA binding domain of p53 show retention of a wild-type *p53* allele.[73] The tumors from those families with functionally null *p53* germline mutations invariably exhibit loss of the remaining wild-type *p53* allele. There is evidence that many *p53* missense mutants can behave in a dominant-negative manner and functionally inactivate wild-type p53 forms.[73] Thus, selective pressure for loss of the wild-type allele in the tumor is reduced. In those LFS patients with a null *p53* allele, it is expected that there would be higher selective pressure for loss of the remaining wild-type allele. Such results in humans would imply that all of the tumors from the *p53*-null heterozygous mice should exhibit loss of the wild-type *p53* allele. In fact, only about half of the p53-null heterozygous mouse tumors do exhibit loss of the wild-type *p53* allele.[73] These tumors tend to arise sooner and are more aggressive than those heterozygote tumors that retain wild-type p53.[74] However, tumors from heterozygous mice with germline missense *p53* mutations invariably retain their wild-type *p53* allele, more consistent with the LFS observations. The retention of wild-type *p53* in the *p53*-null heterozygous tumors indicates that in mice, unlike in humans, reduction of p53 dosage is sufficient to promote tumorigenesis, although loss of both alleles certainly accelerates tumor formation. In humans, functional (via dominant-negative mutant p53 effects) or structural loss of wild-type *p53* seems to be a prerequisite for tumor formation. Such species differences are consistent with generally higher constraints on oncogenic transformation of human cells compared to mouse cells.[75] They are also consistent with the fact that *p53* mutations are relatively rare in spontaneous and carcinogen-induced tumors in mice, in considerable contrast to spontaneous tumors in humans. Thus, humans and mice may harbor some fundamental differences in their requirement for disabling p53 function in the path to tumorigenesis.

The development of mice with a *p53*-null allele, as described above, has been instrumental in our understanding of p53 function in tumorigenesis. However, the majority of individuals with LFS that inherit *p53* mutations inherit missense mutations (>80%). The increased incidence of *p53* missense mutations in LFS patients and in somatic tumors suggests additional oncogenic properties of mutant p53.

Four mouse models have now been generated, using knockin technology, that contain specific missense mutations in *p53*. The first mouse contained the $p53^{R172H}$ mutation, which corresponds to the $p53^{R175H}$ hotspot mutation in human cancers, but expressed low levels of mutant p53 because of an additional splicing abnormality.[76] Nevertheless, heterozygous mice with this mutation developed tumors that were highly metastatic as compared to the rare occurrence of metastasis in $p53^{+/-}$ mice and suggested, for the first time, that *p53* missense mutations could confer a gain of function even when expressed at low levels. The same $p53^{R172H}$ mutation expressed at appropriate levels recapitulated these data and yielded additional insights into the role of *p53* missense mutations.[77,78]

Mice inheriting the $p53^{R172H}$ mutation were studied in 2 different backgrounds.[77,78] In both backgrounds, a metastatic phenotype in heterozygous mutant mice was obvious, although the presence of metastasis did not alter survival. Additionally, in the $129S_4$/Sv background, mice heterozygous for the $p53^{R172H}$ mutation showed a 2-fold increase in the number of osteosarcomas and a slight increase in the number of carcinomas as compared to the $p53^{+/-}$ mice.[78] These data have important implications for human disease in that they indicate that mutant p53 has additional activities not represented by loss of p53, even though both result in loss of p53 transcriptional activity.

Additional insights into the function of missense mutations came from the generation of another p53 mutation, the $p53^{R270H}$ mutation, which corresponds to the human $p53^{R273H}$ hotspot mutation. An important difference between mutations is that the $p53^{R172H}$ mutation represents a conformational mutant, while the $p53^{R270H}$ mutation represents a contact mutant. In the $129S_4$/Sv background, $p53^{R270H}$ heterozygous mice showed increased tumor burden, increased incidence of carcinomas and hemangiomas, and a metastatic phenotype as compared to $p53^{+/-}$ mice. Thus, different *p53* alleles show different tumor spectra. Whether different *p53* mutations give rise to different spectra in humans will be difficult to decipher because of the inherent differences in humans.

The availability of mice with one mutant and one wild-type *p53* allele allowed an evaluation of the dominant-negative nature of mutant p53. The lack of differences in the survival of p53 heterozygous and mutant heterozygous mice indicates that the presence of mutant p53 has no effect on wild-type p53 activity in terms of longevity.[77] Additionally, heterozygous $p53^{R172H}$ mutant mice could not rescue the p53-dependent phenotype of Mdm2-null mice, again suggesting that wild-type p53 was not inactivated by the presence of mutant p53. In contrast, p53-dependent apoptosis in the developing nervous system of $p53^{R172H/+}$ mice resembled that of *p53*-null mice, suggesting that in this case, wild-type p53 was inactivated by mutant p53. Thus, only in some cases does mutant p53 function as a dominant negative. This may be due to the observation that mutant p53 in normal mouse tissues is unstable and, therefore, unable to function as a dominant negative. The stability of mutant p53 in normal LFS samples has yet to be determined.

Lastly, a rare human mutation in *p53* that corresponds to an Arg-to-Pro substitution at amino acid 172 has also been made by knockin of a point mutation.[79] This mutation occurs in human cancers but has not yet been identified in LFS patients. However, this mutation separates the apoptotic from cell cycle arrest functions of p53 and has yielded exciting results. Cells from mice homozygous for the $p53^{R172P}$ mutation are unable to initiate p53-dependent apoptosis and thus resemble p53-null cells. Importantly, $p53^{R172P}$ homozygous mutant cells retain the ability to induce cell cycle arrest and maintain a stable genome. Homozygous mutant mice show a dramatic delay of tumorigenesis as compared to $p53^{-/-}$ mice, suggesting that the ability to suppress the cell cycle is also an important tumor suppressing activity. Thus, the cell cycle arrest function of p53 is also important in the inhibition of tumorigenesis.

The generation of mice with specific missense mutations in *p53* suggests that the inheritance of *p53* mutations as opposed to those mutations that lead to loss of p53 will lead to a worse prognosis. Moreover, different missense mutations may have different effects. Other underlying genetic modifying factors that contribute to the age of onset and the kinds of tumors that develop in LFS patients may also be modeled in mice and offer insight into the human condition.

Medical and Ethical Considerations

Presymptomatic molecular testing for p53 germline mutations in members of LFS kindreds has been met with significant controversy. Because of the variable expressivity, the diverse tumor spectrum, and lack of clear clinical surveillance, preventative, or treatment recommendations, it is unclear how to best manage the detection of a *p53* mutation carrier. It has been suggested that women who carry *p53* mutations should begin screening for breast cancer in their mid-20s, given that the average age of onset is 31 years.[10] Recently, use of PET-CT as a clinical surveillance modality has been reported, identifying presymptomatic lesions in adults,[80] and anecdotal reports of presymptomatic detection of childhood cancers, in particular adrenocortical carcinoma, have also been noted.[81,82] The concept of predictive genetic testing of a child for a disease that may (or may not) occur in young adulthood challenges our perception of the ethics of disclosure of genetic test results, where the potential beneficiary of these results may wish to uphold the right to "not know." Notwithstanding these considerations, presymptomatic and even prenatal genetic testing for p53 is being performed in carefully selected and counseled situations, taking into account the particular balance of beneficence and harm.[83-86] Preimplantation genetic diagnosis has also been advocated, and is available in some jurisdictions, for selection of embryos that do not harbor germline *TP53* mutations.

Although predisposition testing may identify asymptomatic carriers, and facilitate institution of preventive or surveillance programs where available, the following caveats must be considered: 1) the genetic heterogeneity of cancer predisposition; 2) the technical difficulty inherent to gene testing and to test interpretation; and 3) the psychosocial impact of testing. Both variable degrees of penetrance and expressivity for LFS suggest that other genetic events play an important role in defining the particular cancer phenotype of individual members of families. This variability makes predictions of clinical disease and specific susceptible target organs difficult and complicates the design of adequate screening programs.

The technical aspects involved in predisposition gene testing and interpretation are complex. Some tests are only available through research settings, where results are made less immediately available, and confirmation of results is less well controlled than in clinically certified laboratories. Databases are now available to facilitate identification of clinical and research laboratories performing specific genetic tests (e.g., www.genetests.org). Furthermore, such testing, particularly of novel genes, tends to be expensive, and the physician may need to make an extra effort to obtain insurance coverage of testing. Given the complexity, genetic testing should only be undertaken by a physician or genetic counselor fully capable of interpreting these results.

Genetic testing for LFS may have profound psychological and emotional impacts on patients and may be further complicated by relationships with parents and other family members.[87] Issues of the "vulnerable child syndrome" in affected carriers and "survivor guilt" in unaffected, noncarrier siblings raise complex psychosocial concerns that may be beyond the general purview of the pediatric or medical oncologist. Furthermore, lessons from studies in adults have demonstrated that although patients learning about their increased risk of

disease usually do well, they may experience feelings of shock, depression, grief, altered self-esteem, or even guilt. In a study of 135 LFS family members offered genetic testing and counseling, it was observed that greater cancer-specific distress was associated with having a lower quality of life, a higher perceived risk of having a *TP53* mutation, no personal history of cancer, and a greater number of first-degree relatives affected with cancer.[83] Lower perceived self-efficacy in coping with a positive test result was associated with greater cancer worry, higher decisional conflict about p53 testing, and no personal history of cancer. Other similarly designed studies, however, have demonstrated in fact that unaffected individuals in cancer predisposition testing programs are generally accurate in anticipating emotional reactions to test results. However, cancer patients may underestimate their distress after disclosure of positive results; it is these latter individuals who could benefit from intervention strategies.[88] Limited studies in children, parents, and families have yet to clarify the impact of predictive testing for cancer in children.

In an attempt to address these issues, guidelines for testing have been established by both the American Society of Human Genetics[89,90] and the American Society of Clinical Oncology.[91] These guidelines form a useful foundation on which to build practical testing parameters as better defined genotype-phenotype correlations are generated. A recent comprehensive study from France explores the perceptions of 2 groups of genetic service providers for the usage of prenatal diagnosis (PND) and preimplantation genetic diagnosis (PIGD). As parents are now routinely discussing these options in planning future pregnancies, the need to engage a multidisciplinary team in these discussions is key to providing parents and families with the necessary tools to make these ethically challenging decisions.[84,92,93] While some studies suggest the benefits to predictive genetic testing for children are still not substantial, further evaluations from different perspectives will continue to evolve this field.[93,95]

Several recommendations established in 1992 for LFS[96] are still applicable to genetic testing in family cancer syndromes that include children. The quality of information provision on cancer genetics is directly related to the knowledge of professionals and their ability to communicate this to a patient and family, regardless of their specialty.[96] The multidisciplinary approach taken by several groups[92,97] involving pediatric and medical oncologists, clinical geneticists, genetic counselors, psychologists, and ethicists in establishing cancer genetics clinics and programs provides a novel mechanism that should be considered standard to optimize care of these families and advance our understanding of the role of genetics in the etiology and management of LFS.

Declaration of Conflicting Interests

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