Li-Fraumeni Syndrome
Elucidation of the Mechanisms of Cancer Initiation that Began at Hiroshima

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Abstract Although most pediatric oncologists are aware that Li-Fraumeni syndrome (LFS) is characterized by germline p53 mutations, few appreciate that the discovery of this syndrome in 1969 ultimately finds its origins in the detailed epidemiological investigations of cancer in the victims of the atomic bomb at Hiroshima. Both Dr. Robert W. Miller, a pediatrician who became Director of the U.S. National Cancer Institute, and many Japanese pediatricians and paramedical staff from the Atomic Bomb Casualty Commission (ABCC) Pediatrics Study expended enormous efforts to clarify this relationship. The importance of the study of cancer clusters to better understand cancer etiology is derived from their work. We now understand LFS to be a rare autosomal dominantly inherited familial cancer predisposition syndrome characterized by malignancies of both epithelial and mesenchymal origin including soft tissue sarcomas, breast cancer, osteosarcoma, leukemia, brain tumors and adrenocortical carcinoma. In 1990, constitutional mutations of the p53 tumor suppressor gene were shown to be responsible for LFS, a discovery that defined cancer as a genetic disease. Since then, LFS has been the subject of intense clinical and fundamental biological investigation. Recent studies suggest that the types of tumors that develop may in part be determined by the specific p53 mutation, and that murine models of p53 dysfunction accurately reflect the LFS phenotype, providing in vivo models with which to study p53-mediated mechanisms of carcinogenesis. Whereas approximately 70% of LFS is associated with p53 mutations, the cause of the remaining 30% are generally unknown. This unpredictability of genetic penetrance raises numerous ethical issues related to genetic testing and clinical surveillance. This year, 2005, is the 60th anniversary of the bombing of Hiroshima, an appropriate time to review what is known about LFS, the paradigm cancer predisposition syndrome.

Key words: Li-Fraumeni syndrome (LFS), p53, mutation, hereditary cancer predisposition, genetic diagnosis

I. Prologue

"Don't study rare cancers because they have no public-health significance, and don't study genetics because it can't be fixed."

"On 20 November 1953, I arrived in Hiroshima via pediatrics, radiation biology, and troop train. Three years earlier, upon completing my training in pediatrics, I did not feel finished. When I heard that John Morton, Professor of Surgery at the University of Rochester, was to spend six months as interim director of the Atomic Bomb Casualty Commission (ABCC), it seemed that my own short assignment there would add clinical experience to what I had already learned. I was interviewed by Grant Taylor, the departing director of ABCC, and agreed to a one-year term. Soon after, I was in Honolulu for an overnight stay in a $4-a-night hut in a jungle (now the International Market) across the street from its parent, the Moana Hotel, then to Wake Island and Tokyo by Pan American sleeper DC-6, the berth was in what is now known as the overhead luggage rack. Next, a night at the marvelous Imperial Hotel, which was designed by Frank Lloyd Wright and razed many years ago, in Tokyo, and from there to Hiroshima by the U.S. troop train. Being in Japan at that time was like being seven years old again and seeing many things for the first time. It was enchanting, and the work was fascinating. We examined about 20 children a day in the clinic, as Japanese children’s songs played on a tape recorder in the waiting room."

II. Introduction

Pediatric oncologists today are interested in hereditary cancer predisposition syndromes since they tend to develop in children or young adults and because the course of the disease is closely associated with the interaction of genetic background and environmental cofactors. This was not the case at the time of the atomic bomb. Hereditary syndromes had occasionally been described as medical oddities. While physicians noted common medical abnormalities and speculated on possible relationships, the lack of fundamental understanding of epidemiological links made further definitive characterization of phenotypes difficult.

Miller submitted a report to a pediatric research society in the U.S. describing findings among radiation-exposed children a decade after the bombs, but the report was not accepted. However shocked he had been by his observations, they did not seem to be of broader relevance to pediatric research at that time. He had a similar experience in Japan. As a distinguished foreign visitor he was scheduled to speak to the Japan Medical Association on the late effects of the bombs on children, but virtually no one came to listen. They went instead to a lecture on ekiri, a common life-threatening diarrheal disease thought to be unique to Japanese children.

After his experiences in Japan, Miller decided to concentrate on the problem of radiation effects on children from an epidemiologic perspective, an unpopular discipline among physicians of the time. He went back to the U.S. with a Japanese bride, as a pediatrician specializing in the biological effects of radiation. At the National Cancer Institute (NCI), he investigated the epidemiology of congenital anomalies, starting with the association of Wilms’ tumor with congenital malformations. Then, in 1964 Miller and Fraumeni described the association between Wilms’ tumor and the rare congenital anomalies aniridia and hemihypertrophy. He then pursued studies to determine the extent to which childhood cancers in siblings aggregate by studying death certificates for all 21,659 children under 15 years of age who died of cancer in the U.S. between 1960 and 1964. He also compared cancer mortality in childhood in the U.S. and Japan. From their cumulative epidemiological data, Fraumeni and Miller discovered an intriguing relationship between adrenocortical tumors, brain tumors, hemihypertrophy, and other childhood cancers.

Following upon these ground-breaking cancer epidemiology studies, in 1969, Li and Fraumeni reported observations from a retrospective analysis of the family histories of 648 children who had died of rhabdomyosarcoma. They initially reported an inherited cancer predisposition syndrome on the basis of four families in which two cases of sarcoma occurred in early life. These four families all had striking histories of breast cancer and other tumors. In this seminal paper that provided the original definition of the Li-Fraumeni syndrome (LFS), they concluded with the remark that ‘laboratory studies are being conducted on surviving members of the families in an effort to evaluate genetic factors and viruses in the cause of the neoplasms’.

An entirely different line of study led to the discovery of the p53, which was initially identified in 1979 as a cellular protein bound by the simian virus 40 (SV40) large T antigen. Initially thought to be a tumor antigen, p53 was subsequently characterized as an oncogene because of its apparent ability to cooperatively induce cellular transformation. However, in the late 1980’s, evidence mounted that indicated p53 was in fact a tumor suppressor, mutated in a wide spectrum of human malignancies, and capable of inducing cell cycle arrest and growth inhibition. p53 is now popularly known as the “Guardian of the Genome”, occupying a singularly prominent place in our understanding of human cancer.

In 1990, Malkin and colleagues, investigating the relationship between LFS and p53, discovered that germ line p53 mutations were causally associated with LFS. This observation supported the notion of cancer as a genetic disease. Subsequently, an ever-growing body of information describing the complex relationship between p53 and LFS has accumulated.

III. Clinical Features and Definitions

1. Cancer type

The most commonly observed tumors in LFS include osteosarcomas, soft tissue sarcomas, premenopausal breast cancer, brain tumors, adrenocortical tumors, and acute leukemias. These are generally inherited in an autosomal dominant pattern. Numerous other tumors including early-onset gastric and pancreatic carcinomas, and other pediatric cancers have been observed with increasing frequency in LFS kindreds, and multiple synchronous or metachronous malignancies occur in greater than 30% of patients. Various less stringent definitions have been proposed, including that of the Li-Fraumeni-like syndrome (LFS-like) to account for the variability in identification of germ line p53 mutations in individuals or families who do not fit the classical definition. The definitions of LFS and LFS-like are summarized in
Table 1. The Clinical definitions of LFS and LFS-like

<table>
<thead>
<tr>
<th>LFS-like</th>
<th>LFS</th>
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| A proband with a sarcoma diagnosed before 45 years of age AND | Classic LFS is defined by the following criteria:

A proband with a sarcoma diagnosed before 45 years of age AND
A first-degree relative with any cancer under 45 years of age AND
A first- or second-degree relative with any cancer under 45 years of age or a sarcoma at any age | A first-degree relative with any cancer under 45 years of age AND
A first- or second-degree relative with any cancer under 45 years of age or a sarcoma at any age
Birch’s definition of LFS-like:⁵⁰
A proband with any childhood cancer or sarcoma, brain tumor, or adrenal cortical tumor diagnosed before 45 years of age AND
A first- or second-degree relative with a typical LFS cancer (sarcoma, breast cancer, brain tumor, adrenal cortical tumor, or leukemia) at any age AND
A first- or second-degree relative with any cancer under the age of 60 years
Eeles' definition of LFS-like:⁵¹
Two first- or second-degree relatives with LFS-related malignancies at any age

²⁰ Six codons of p53 (175, 245, 248, 249, 273 and 282) denoted hot spots account for nearly 25% of reported sporadic missense mutations (Fig. 1). While other mutations at codons 258 and 252 were also reported in the original LFS reports,¹¹ numerous subsequent studies indicate that both germline and sporadic p53 mutations occur throughout the gene, and are not confined to the DNA binding domain. Germline p53 mutations are found in approximately 70% of LFS individuals; 30% of LFS and the majority of LFS-like families do not have detectable germline mutations within the coding region of p53. Whether this lack of 100% concordance between the genotype and apparent LFS phenotype is due to incomplete gene analysis, causative links with other genes, or misidentification of the clinical syndrome has not been clearly established.

An intriguing association between germline p53 mutations and cancer is found in the setting of childhood adrenocortical carcinoma (ACC) that has an incidence rate of only 0.3/million children, yet is not infrequently observed in LFS families. In a series of individuals with childhood ACC, 35 of 36 children were found to have the identical germline p53 mutation: R337H at codon 337 in which arginine is substituted with histidine.²⁴ Varley and colleagues investigated the frequency of germline p53 mutations in a cohort of children with ACC or adrenal adenomas unselected for family history and found that 22/25 had germline p53 mutations at either codons 152 or 158. Additionally, all 12 LFS and LFS-like families that they have studied in which there is a case of ACC have a germline p53 mutation.²⁵ Thus, ACC represents a definitive diagnostic feature in families with LFS. In particular, germline mutation at codons 337, 152 and 158 are strikingly associated with ACC. While the biochemical characteristics of codon 152- and 158-associated ACCs are not well-characterized,
the codon R337H mutation confers a pH-dependent instability of the mutant p53 tetramer.26 The authors propose that the vastly increased risk of children with this mutation to develop ACC is a consequence of the increased pH levels in apoptotic adrenal cortex cells during the pre- and post-natal development of the adrenal gland. To date, no other characteristic genotype: phenotype correlation has been observed, suggesting perhaps that mutations in other domains of the gene do not confer specific cancer phenotypes.

Although germline p53 mutations account for the majority of LFS, there are individuals and families with germline p53 mutations who do not meet the clinical definitions of LFS (Table 2). While germline p53 mutations are thought to account for less than 1% of total cases of early-onset breast cancer,11,27 the frequency of certain apparently sporadic childhood cancers in germline p53 carriers is more prevalent. A series of reports of individuals with other malignancies (irrespective of family history) have reported the following p53 mutation frequencies: 2-10% of childhood brain tumors,28,29 75-100% of childhood ACC,14,24 2-3% of osteosarcomas,30 9% of rhabdomyosarcomas,31 7-20% of multiple primary tumors occurring at early ages.32 These individuals frequently exhibit some clinical features of LFS; their tumors have an early age of onset and they often have multiple primary tumors.32-34 Germline p53 mutations can also predispose to a wide range of tumor types not observed in LFS including; non-Hodgkin lymphoma, neuroblastoma, and lung, colon, and gastric carcinomas.32,35 It is even possible that the frequencies are higher than those reported, as most of these studies were performed before comprehensive analysis of the gene was made possible with high-throughput automated sequencing and complete gene mutation analysis. Nevertheless, it is apparent that in certain sub-populations of cancer patients—particularly those with early-onset childhood cancers—consideration of p53 analysis should be given in determination of etiology of the disease, and potential opportunities for presymptomatic screening of at-risk family members.

The genetics of p53 transmission in the germline has been evaluated in animal models. Several mouse models carrying p53 mutations have been generated, and their cancer phenotypes examined. While p53-deficient mice generated by Donehower, et al in 1992 develop a spectrum of malignancies somewhat resembling those observed in LFS, the model that most closely approximates the LFS is that with a heterozygous p53 mutation (mt/+).36,37 Both the groups of Jacks and Lozano generated heterozygous p53 (mt/±) mice in which the mutations corresponded to the human hot spots p53 missense mutations occur in the DNA-binding domain. Codons 248, 273, 175, 245, 249 and 282 are the sites of hot spots missense mutations and the frequency of mutations is high in this order. Numbers indicate residue number.

The p53 protein consists of 393 amino acids and is commonly divided into three functional domains. Most of the interactions between p53 and its target proteins take place in the DNA-binding domain of p53. The C-terminal end contains nuclear localization and export signals (NLS and NES, respectively), a regulatory domain and the tetramerization domain. The N-terminal portion consists of the transactivation domain that is required for transactivation activity and interacts with various transcription factors. The SH3 domain is a proline-rich domain, which protects p53 from degradation. 95.1% of p53 missense mutations occur in the DNA-binding domain. Codons 248, 273, 175, 245, 249 and 282 are the sites of hot spots missense mutations and the frequency of mutations is high in this order. Numbers indicate residue number.

![Fig. 1 p53 structure and six-hot spots for missense mutations](image-url)
that development of the LFS phenotype presumably requires the combination of one wild-type p53 with a missense mutation in the other allele. This may account for the extraordinarily rare occurrence of a classic LFS phenotype in the absence of a readily identifiable heterozygous germline p53 mutation in humans.

2. Chk2

Chk2 is a protein kinase acting downstream of ATM (ataxia telangiectasia mutated) and regulating cell cycle arrest (see Fig. 2). The study of murine embryonic stem cells deleted for the Chk2 gene confirmed that Chk2 kinase and p53 act together in the DNA damage response pathway, providing a molecular rationale for the involvement of mutations in both genes in LFS. Somatic Chk2 mutations have been reported in a variety of tumor types, although they appear to be commonest in osteosarcomas. While germline Chk2 mutations have been identified in a few families with classic LFS and several LFS-like families, it is now thought more likely that Chk2 is a low penetrant tumor-suppressor gene in breast cancer. In particular, the 1100delC mutation of the Chk2 gene has been reported to confer low-penetrance breast cancer susceptibility.

3. MDM2

As a negative regulator of p53, increased expression of MDM2 attenuates the p53 growth regulatory pathway (see Fig. 2). Bond and colleagues investigated whether naturally occurring polymorphisms in MDM2 might influence cancer susceptibility. They identified a single-nucleotide polymorphism (SNP)-SNP309 (T > G) that was present at relatively high frequency in 50 healthy individuals. They first determined that LFS was characterized by higher levels of SNP309 / MDM2 as well as a weak response to DNA-damage. Furthermore, these individuals developed tumors at a younger age and were likely to subsequently develop multiple primary tumors. The SNP309 / MDM2 also affects the risk of sporadic cancers; soft tissue sarcomas were diagnosed on average 12 years earlier in individuals with this variant.

4. The other molecules

p16(INK4a), p14/p19(ARF), PTEN, p63,
Bel10,\textsuperscript{45} and Chk1\textsuperscript{46,47} are predicted to be candidates potentially involved in the generation of the cancer phenotype of LFS and LFS-like syndromes. Mutations in p16 (INK4a) are a major contributor to carcinogenesis and its inactivation is observed in up to 26\% of all malignancies.\textsuperscript{55} Germline mutations of p16 (INK4a) can result in the hereditary predisposition to the development of melanoma and pancreatic cancer.\textsuperscript{56,57} Of 103 germline samples taken from LFS and LFS-like family members, seven missense mutations were detected. Among these, five were a common exon 2 polymorphism, and the other two were functionally neutral.\textsuperscript{48} p14/p19(ARF) is a tumor suppressor gene derived from the same locus as p16(INK4a). Germline mutations of p14/p19(ARF) were found in 1 of 15 LFS-like families.\textsuperscript{49} On the other hand, no germline mutations of either p16 (INK4a) or p14/p19(ARF) were identified in 16 LFS and LFS-like samples in another cohort of families.\textsuperscript{50} To date, no coding germline mutations have been detected in PTEN, p63, Bel10 or Chk1.\textsuperscript{45,56-54}

V. Genetic Counseling

The molecular and clinical study of LFS/LFS-like families leads to a wide variety of medical, psychological, and familial issues making effective genetic counseling essential.\textsuperscript{58,59} The medical, psychosocial, and ethical consequences of identifying at-risk individuals through cancer risk assessment with or without molecular genetic testing are profound. If the at-risk individual is older than age 50 years and has never had cancer, this risk gradually decreases with age. However, the unpredictability of the cancer phenotype, onset of tumors, or likelihood of multiple cancer formation makes effective risk counseling difficult. The risk of developing cancer in childhood (before 18 years of age) approaches 40\%, which necessitates a careful evaluation of benefits to presymptomatic testing of children who are relatives of known mutant p53 gene carriers. The elements required for informed consent for cancer genetic testing are listed in Table 2.

In addition to the complex issues related to screening of affected children, the potential for prenatal screening has also been raised. Avigad and colleagues discussed the case of a ‘classic’ LFS family in which the parents of the proband had opted for termination of the pregnancy for two fetuses harboring the same germline p53 mutation found in their son who suffered from rhabdomyosarcoma (Fig. 3). The authors concluded that children with primary tumors belonging to the LFS constellation should be considered for germline screening for p53 mutations as well as genetic counseling by a multidisciplinary team, and that screening of asymptomatic siblings or prenatal testing at least be considered. These measures may prove psychologically beneficial to high-risk families whether family members are found to be carriers or mutation-free.\textsuperscript{60} Although the decision of the parents in this situation must always be respected, access to a multidisciplinary team including pediatricians, pediatric oncologists, psychologists, ethicists, and genetic counselors, must be made available in an attempt to alleviate the enormous burden on the parents. The parents may feel guilty as a consequence of having transmitted the defective gene and they will be concerned about the unborn child’s right to ‘not know’ their genetic destiny. If the parents had decided to continue the pregnancy, then it would be incumbent on physicians to utilize effective relatively non-invasive clinical screening and surveillance studies to increase the likelihood of early detection of tumors as much as possible.\textsuperscript{61} The difficulties related to the clinical application for a genetic test apply not only to LFS but also to several other hereditary syndromes with a cancer phenotype. Thus, the lessons learned from the studies of LFS will apply to principles of clinical surveillance for children and young adults with a spectrum of cancer predisposition disorders.

**Table 2** Frequency of germline p53 mutations in LFS, LFS-like, and sporadic cancers

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Frequency of germline p53 mutations (%)</th>
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<tbody>
<tr>
<td>LFS with adrenocortical cancer</td>
<td>75-100</td>
</tr>
<tr>
<td>LFS</td>
<td>50-85</td>
</tr>
<tr>
<td>Sporadic adrenocortical cancer</td>
<td>40-70</td>
</tr>
<tr>
<td>LFS-like</td>
<td>10-30</td>
</tr>
<tr>
<td>Sporadic rhabdomyosarcoma</td>
<td>9</td>
</tr>
<tr>
<td>Multiple cancer at early ages (non-LFS)</td>
<td>7-20</td>
</tr>
<tr>
<td>Sporadic second neoplasms</td>
<td>5-15</td>
</tr>
<tr>
<td>Sporadic brain tumors</td>
<td>2-10</td>
</tr>
<tr>
<td>Sporadic osteosarcoma</td>
<td>2-3</td>
</tr>
<tr>
<td>Early onset breast cancer</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>
VI. Epilogue

'I sought to use all of the information I had acquired—about pediatrics, radiation effects, and epidemiology—in the most clinical way possible. My lack of knowledge about cancer etiology may have been an advantage: I had no preconceptions about it. Very little attention had been given to childhood cancer etiology, and we made quick progress by means of descriptive studies of mortality (by age and race in particular) and by finding associations of certain cancers with specific birth defects. The syndromes we delineated or studied have since led to the identification of tumor-suppressor genes: Wilms' tumor of the kidney and congenital absence of the iris of the eye, neurofibromatosis types 1 and 2, trilateral retinoblastoma (involving both eyes and the pineal gland), and the Li-Fraumeni cancer syndrome. Studies of these rarities have led to understanding of how substantial proportions of common cancers develop—cancers of the breast, colon, bone, and lung, among others. These findings belied the advice given to us 25 years ago: don't study rare cancers because they have no public-health significance, and don't study genetics because it can't be fixed.


The practice of medicine is based on the observations of generations of physicians. It is a fundamental requirement for the physicians to examine each patient carefully and record the symptoms in as much detail as possible. The 'history' of the Li-Fraumeni syndrome is just such an example of the remarkable contributions to our understanding of the biological and clinical understanding of human cancer through the

Table 3 The elements of informed consent for cancer genetic testing

| 1. | What the test is intended to do, i.e., determine whether a mutation can be detected in a specific cancer susceptibility gene |
| 2. | What can be learned from both a positive and negative test, including information on the magnitude of health risks associated with a positive test, as well as the risks that may remain even after a negative test |
| 3. | The possibility that no additional risk information will be obtained after testing, or that the test will result in a finding of unknown significance (e.g., a polymorphism) that may require further studies |
| 4. | The options for approximation of risk without genetic testing, e.g., using empiric risk tables for breast cancer given differing family histories |
| 5. | The risk of passing a mutation on to children |
| 6. | The importance of notification of family members that they may share a hereditary risk for cancer with every effort made to assist in contacting of family members and providing them access to counseling and testing |
| 7. | The medical options and limited proof of efficacy for surveillance and cancer prevention for individuals with a positive test, as well as the accepted recommendations for cancer screening even if genetic testing is negative |
| 8. | The technical accuracy of the test; the sensitivity and specificity of the analytic methodology |
| 9. | The risks of psychological distress and family disruption, whether a mutation is found or not found |
| 10. | The risk of employment and/or insurance discrimination following disclosure of genetic test results, and the level of confidentiality of results compared to other medical tests and procedures |
| 11. | The risks that non-relatedness of family members will be discovered, and how this information will be disclosed (or not disclosed) |
| 12. | The fees and costs of testing, including the laboratory test and the associated consultation by the health professional providing pretest education, results disclosure, and follow-up, and the costs of preventive procedures, which may not be covered by third party payers |

marriage of astute clinical observation with fundamental discoveries in human cancer biology.

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