sequences. However, since the reversion rate in retroviral replication of such a 4-bp insertion is lower than 10^{-7} per insertion (*5*), this explanation is unlikely. Different markers in the two U5 regions may also result from mismatch repair of either the 5′ U5 or the 3′ U5 region. If the 5′ U5 region experienced the repair and resulted in a different marker than the 3′ U5 region, then the minus strand transfer had to be intermolecular (from WH204 RNA to WH13 RNA) to form the 3′ LTR. Suppose the minus strand strong stop DNA synthesis of WH13 RNA never initiated. As a result u5 and pbs were intact. Then it was possible for the minus strand DNA synthesis to continue and copy the U5 marker of WH13 (Bam HI). After completion of DNA synthesis and repair, the Bam HI marker remained in the 5′ U5 and resulted in different markers in the two U5 regions. It is also possible that the 3′ U5 region was repaired to a different marker. Then the minus strand strong stop transfer had to be intramolecular (from WH13 to WH13) to result in the 5′ WH13-like LTR. Suppose a strand displacement event occurred at the end of the 3′ LTR and formed a mismatch at the 3′ U5: the minus strand DNA contained a Bam HI site and the plus strand contained a Cla I site from the displaced fragment. After repair, the Cla I marker remained and resulted in the two different U5 markers. It is most likely that the different markers in the two U5 sequences were generated from mismatch repair of one of the LTR's. However, it is not clear which pathway generated this recombinant provirus.

27. Fisher's exact test was calculated with 21 recombinants. The recombinant with two different markers in the U5 was not included because it is not clear which type of minus strand transfer was used. Calculation including this sample was also performed, and the result indicated the distribution still is not random.
28. H. M. Temin, *Trends Genet.*, in press.
29. We thank Rebecca Wisniewski, Bonnie Fritz, and Kevin Krebsbach for technical assistance; N. Drinkwater, W. Sugden, and M. Susman for critical reading of the manuscript; M. Hannink, K. Iwasaki, V. Pathak, and G. Pulsinelli for discussion and helpful comments. Supported by Public Health Service grants CA-22443 and CA-07175 from the National Cancer Institute; a postdoctoral fellowship from the American Cancer Society (W.-S.H.); and an American Cancer Society Research Professorship (H.M.T.)

13 June 1990; accepted 19 September 1990

Germ Line p53 Mutations in a Familial Syndrome of Breast Cancer, Sarcomas, and Other Neoplasms

DAVID MALKIN, FREDERICK P. LI, LOUISE C. STRONG, JOSEPH F. FRAUMENI, JR., CAMILLE E. NELSON, DAVID H. KIM, JAYNE KASSEL, MAGDALENA A. GRYKA, FARIDEH Z. BISCHOFF, MICHAEL A. TAINSKY, STEPHEN H. FRIEND*

Familial cancer syndromes have helped to define the role of tumor suppressor genes in the development of cancer. The dominantly inherited Li-Fraumeni syndrome (LFS) is of particular interest because of the diversity of childhood and adult tumors that occur in affected individuals. The rarity and high mortality of LFS precluded formal linkage analysis. The alternative approach was to select the most plausible candidate gene. The tumor suppressor gene, p53, was studied because of previous indications that this gene is inactivated in the sporadic (nonfamilial) forms of most cancers that are associated with LFS. Germ line p53 mutations have been detected in all five LFS families analyzed. These mutations do not produce amounts of mutant p53 protein expected to exert a trans-dominant loss of function effect on wild-type p53 protein. The frequency of germ line p53 mutations can now be examined in additional families with LFS, and in other cancer patients and families with clinical features that might be attributed to the mutation.

IN 1969, LI AND FRAUMENI REVIEWED MEDICAL RECORDS and death certificates of 648 childhood rhabdomyosarcoma patients and identified four families in which siblings or cousins had a childhood sarcoma (*1*). These four families also had striking histories of breast cancer and other neoplasms, suggesting a new familial cancer syndrome of diverse tumors (Li-Fraumeni syndrome; LFS). Recently completed prospective studies have confirmed the high risk in family members of the tumor types that comprise LFS (*2*). Since the original description of the syndrome, systematic studies and anecdotal reports have confirmed its existence in various geographic and ethnic groups (*3*). The spectrum of cancers in the syndrome (Table 1) has been determined to include breast carcinomas, soft tissue sarcomas, brain tumors, osteosarcoma, leukemia, and adrenocortical carcinoma. Possible component tumors of LFS are melanoma, gonadal germ cell tumors, and carcinomas of the lung, pancreas, and prostate (*4, 5*). These diverse tumor types in family members characteristically develop at unusually early ages, and multiple primary tumors are frequent.

To test the hypothesis that the Li-Fraumeni syndrome has a genetic etiology, Williams and Strong (*5*) applied segregation analysis and demonstrated that the observed cancer distribution in families best fit a rare autosomal dominant gene model. This model also predicted that the probability, for the families at risk, of developing any invasive cancer (excluding carcinomas of the skin)

D. Malkin, C. E. Nelson, D. H. Kim, J. Kassel, M. A. Gryka, and S. H. Friend are in the Division of Molecular Genetics, Massachusetts General Hospital Cancer Center, MGH East, Charlestown MA 02129. F. P. Li and C. E. Nelson are in the Clinical Epidemiology Branch, Division of Cancer Etiology, NCI, NIH, Bethesda MD, 20892 and in the Division of Biostatistics and Epidemiology, Dana-Farber Cancer Institute, Boston, MA, 02115. L. C. Strong is in the Division of Pediatrics, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030. J. F. Fraumeni, Jr., is in the Epidemiology and Biostatistics Program, Division of Cancer Etiology, NCI, NIH, Bethesda MD, 20892. F. Z. Bischoff and M. A. Tainsky are in the Department of Tumor Biology, University of Texas M. D. Anderson Cancer Center, Houston TX, 77030. S. H. Friend is also in the Division of Hematology-Oncology, The Children's Hospital, Dana-Farber Cancer Institute, Department of Pediatrics, Harvard Medical School, Boston MA, 02114.

*To whom correspondence should be addressed.

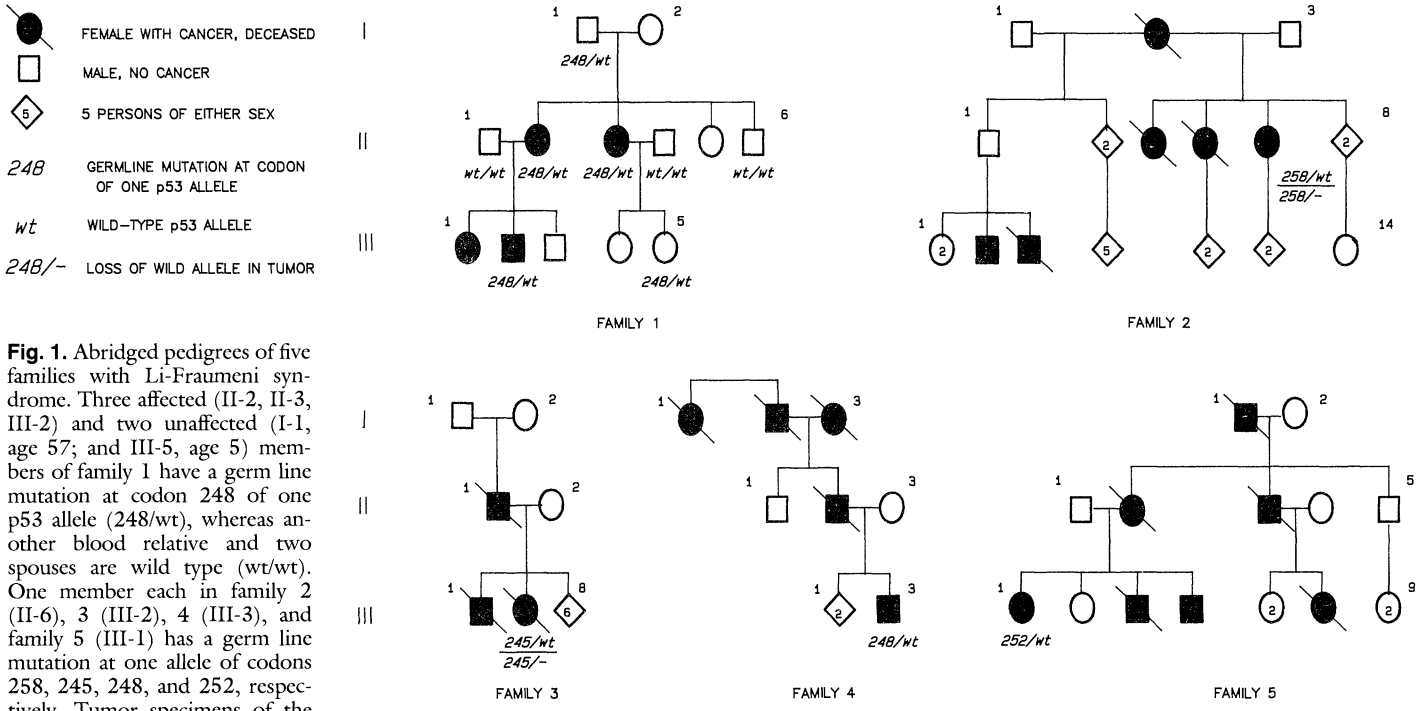

Fig. 1. Abridged pedigrees of five families with Li-Fraumeni syndrome. Three affected (II-2, II-3, III-2) and two unaffected (I-1, age 57; and III-5, age 5) members of family 1 have a germ line mutation at codon 248 of one p53 allele (248/wt), whereas another blood relative and two spouses are wild type (wt/wt). One member each in family 2 (II-6), 3 (III-2), 4 (III-3), and family 5 (III-1) has a germ line mutation at one allele of codons 258, 245, 248, and 252, respectively. Tumor specimens of the single cases in families 2 and 3 show loss of the remaining wild-type allele (258/- and 245/-, respectively). In family 1, II-2 developed unilateral breast carcinoma at age 30; II-3, bilateral breast carcinoma at 28; III-1, soft tissue sarcoma at age 1 and osteosarcoma at age 8; and III-2, brain tumor at age 5. In family 2, I-2 developed carcinoma of ampula of Vater at age 47 and bladder carcinoma at age 53; II-4, ovarian germ cell carcinoma at age 16; II-5, soft tissue sarcoma at age 21; II-6, unilateral breast carcinoma at age 34; III-3, osteosarcoma at age 20; III-4, brain tumor at age 16. In family 3, II-1, developed soft tissue sarcoma at age 58; III-1, osteosarcoma at age 11; and III-2, osteosarcoma at age 19. In family 4, I-1 developed bilateral breast carcinoma at age 68; I-2, melanoma at age 54; I-3, uterine carcinoma at age 66; II-2, melanoma at age 45 and brain tumor at age 52; and III-3, soft tissue sarcoma at age 5 and osteosarcoma at age 21. In family 5, I-1, developed a brain tumor at age 26; II-2, bilateral breast carcinoma at age 27; II-3, brain tumor at age 38; III-1, soft tissue sarcoma at age 2, osteosarcoma at age 15, and unilateral breast carcinoma at age 32; III-3, osteosarcoma at age 22; III-4, leukemia at age 17, and osteosarcoma at age 18; and III-7, brain tumor at age 9.

reaches almost 50 percent by age 30, when only 1 percent of the general population has developed cancer (*6*). More than 90 percent of the gene carriers would develop cancer by age 70. While the syndrome has thus been characterized statistically, identification of the gene and the mechanisms for the striking cancer predisposition has been elusive.

Difficulties in cloning the LFS gene arose from a number of restrictions placed on traditional methods. The accumulation of informative tissue specimens and data has been hampered by the rarity of the syndrome, by the high mortality among affected individuals, and by lack of an unambiguous definition of the syndrome. In addition, the cancers in relatives without the gene often cannot be distinguished from cancers associated with the gene. Linkage studies and searches for constitutional karyotypic alterations in patients with LFS have failed to specify the chromosomal region on which to focus. Furthermore, these families do not have any precancerous conditions or associated malformation syndromes such as aniridia, which was useful in mapping the Wilms tumor gene (WT1) to chromosome 11p13 (7). Because of these difficulties, we targeted potential candidate genes for study.

The class of genes most strongly associated with familial tumors has been the tumor suppressor genes. The first tumor suppressor gene to be cloned, the retinoblastoma gene (Rb) (*8*), seemed an unlikely candidate for a germ line mutation in LFS because retinoblastoma has not been observed in these families. The second tumor suppressor gene to be recognized was p53, located on the short arm of chromosome 17, band p13 (*9*). Inactivating mutations of p53 have been associated with sporadic osteosarcomas, soft tissue sarcomas, brain tumors, leukemias, and carcinomas of the lung and breast (*10, 11*). Together, these tumors also account for more than half of the cancers in selected series of LFS families. Furthermore, transgenic mice carrying a mutant p53 gene have an increased incidence of osteosarcomas, soft tissue sarcomas, adenocarcinomas of the lung, and adrenal and lymphoid tumors (*12*). These tumors all have been noted in families with LFS. We therefore decided to test whether families with LFS carry germ line p53 gene mutations.

Germ line p53 gene mutations in LFS families. Skin biopsies were obtained from three affected and five unaffected relatives in family 1 (see Fig. 1). Their fibroblasts were grown in short-term culture, and DNA was extracted by standard techniques (*13*). The polymerase chain reaction (PCR) was used to amplify the genomic region encoding exons 5 through 8 of the p53 gene (*14*). This segment contains a majority of the conserved domains and most of the mutations found in sporadic tumors (*10, 15*). The primers used for amplification were designed to contain Eco RI sites at their 5′ ends so that the 1685-bp amplified fragment could be subcloned for sequencing. Multiple primers were then used to sequence the entire genomic region on both strands (*16*). Each sequencing reaction was repeated on multiple clones. Three members in family 1 (II-1, II-4, and II-6) have the wild-type p53 sequence in six out of six clones (Table 2). These individuals are considered to carry two wild-type p53 alleles and have no evidence of tumors. DNA from separate clones representing the five other family members contained both wild-type p53, and p53 with a C to T mutation at the first nucleotide of codon 248. These individuals are considered to carry both a wild-type and a mutant p53 allele in their fibroblasts. Even though several polymorphisms have been identified in the p53 gene (*17*), none has been detected in the five conserved domains (*10, 11, 15, 18*). These findings are derived from the sequencing of the p53 gene in more than 150 samples of the DNA extracted from

constitutional cells (*10*). The codon 248 mutation, which changes an arginine to tryptophan, occurs within conserved region IV that spans codons 234 to 258 (boxed area, Fig. 2). It has been shown that it is not a polymorphism; in fact, it is the most common p53 mutation in sporadic tumors studied to date (*10, 11, 18*). The three affected individuals tested in this family have the codon 248 mutation. The unaffected grandfather (I-2) and his granddaughter (III-5) also carry the codon 248 mutation and are carriers predicted to be at high risk of developing cancer.

Mutations in the p53 gene are documented to occur in the establishment of cells in long-term culture (*19, 20*). Thus, during in vitro passage of the LFS fibroblasts, the p53 gene might have acquired changes not present in the germ line. Although it is extremely unlikely that all five fibroblast lines acquired the codon 248 mutation, we collected blood from two of the individuals in this family (II-2 and II-3) who have breast cancer and also one mutant p53 allele in their fibroblasts. DNA was extracted from their lymphocytes after four passages in tissue culture. Lymphocytes from both sisters have a C to T mutation at the first nucleotide of codon 248 (Table 2). This is the same mutation found in fibroblasts of these patients.

To support the hypothesis that this p53 mutation represents a germ line mutation and not artifact of PCR, we studied segregation of the p53 chromosome region in family 1. We used YNZ22.1, a highly polymorphic DNA sequence on chromosome 17p that

Table 1. Cancers in 43 families with Li-Fraumeni Syndrome (LFS), by tumor type and age at diagnosis. All families were ascertained through a proband with sarcoma, who is excluded from the tabulations.

Tumor type	Age at diagnosis (years)*			
	0–14	15–44	>44	All ages
Component tumors of LFS				
Breast carcinoma	0	49	11	60
Soft tissue sarcoma	13	12	4	29
Brain tumors	12	15	1	28
Osteosarcoma	6	6	2	14
Leukemia	8	4	2	14
Adrenocortical carcinoma	5	0	0	5
Possible component tumors of LFS				
Lung carcinoma	0	7	12	19
Prostate carcinoma	0	0	8	8
Pancreas carcinoma	0	1	6	7
Melanoma	0	1	2	3
Other tumors				
Colorectal carcinoma	1	3	4	8
Lymphoma	0	5	1	6
Stomach carcinoma	0	3	1	4
Other	5	13	8	26
All cancers				
	50	119	62	231

*Only first cancer was counted in patients with multiple tumors.

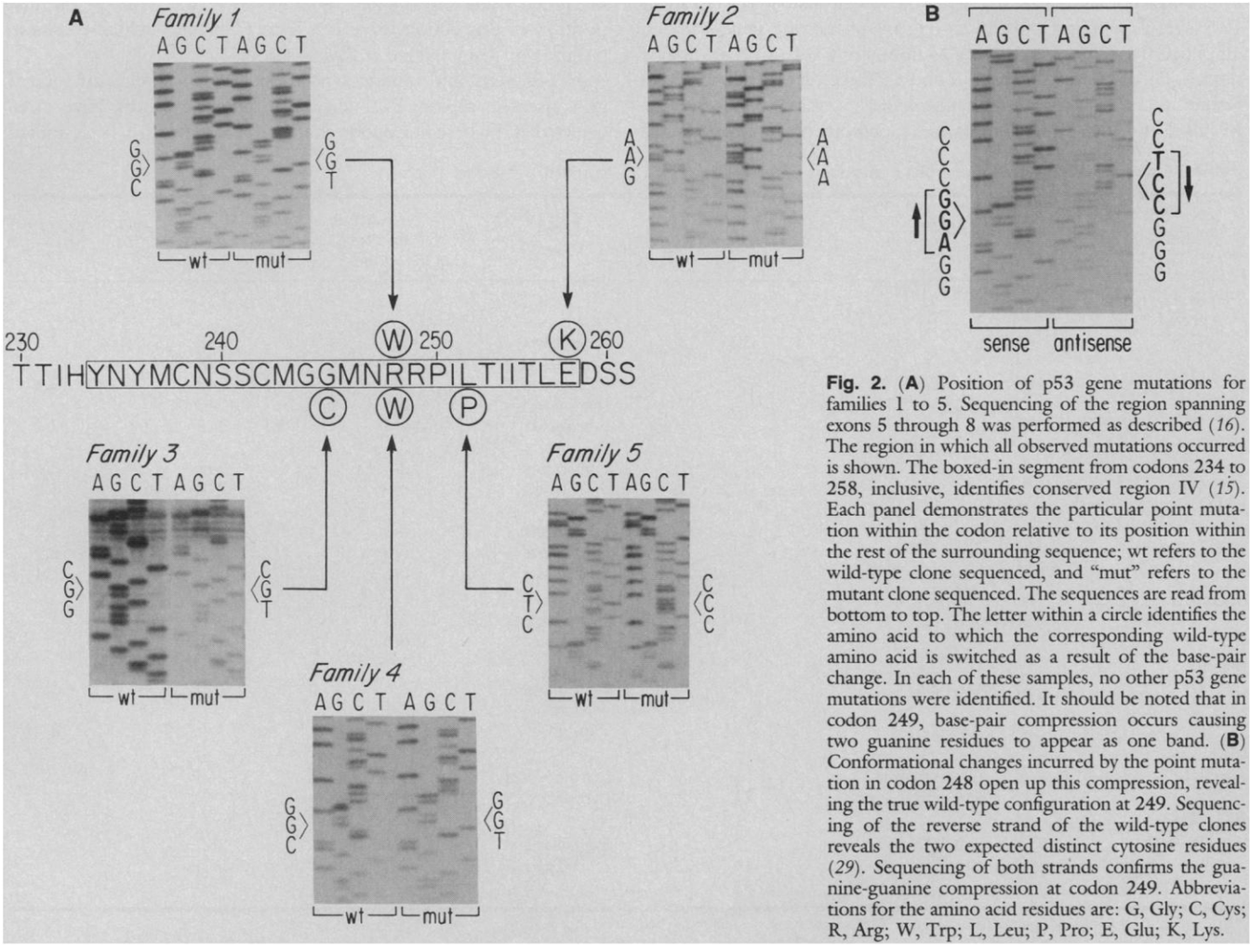

Fig. 2. (**A**) Position of p53 gene mutations for families 1 to 5. Sequencing of the region spanning exons 5 through 8 was performed as described (*16*). The region in which all observed mutations occurred is shown. The boxed-in segment from codons 234 to 258, inclusive, identifies conserved region IV (*15*). Each panel demonstrates the particular point mutation within the codon relative to its position within the rest of the surrounding sequence; wt refers to the wild-type clone sequenced, and "mut" refers to the mutant clone sequenced. The sequences are read from bottom to top. The letter within a circle identifies the amino acid to which the corresponding wild-type amino acid is switched as a result of the base-pair change. In each of these samples, no other p53 gene mutations were identified. It should be noted that in codon 249, base-pair compression occurs causing two guanine residues to appear as one band. (**B**) Conformational changes incurred by the point mutation in codon 248 open up this compression, revealing the true wild-type configuration at 249. Sequencing of the reverse strand of the wild-type clones reveals the two expected distinct cytosine residues (*29*). Sequencing of both strands confirms the guanine-guanine compression at codon 249. Abbreviations for the amino acid residues are: G, Gly; C, Cys; R, Arg; W, Trp; L, Leu; P, Pro; E, Glu; K, Lys.

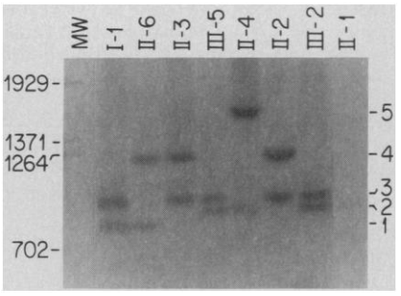

Fig. 3. DNA (Southern) gel blot analysis. Hybridization of the variable number tandem repeat (VNTR) polymorphic probe YNZ22.1 to genomic DNA from the eight members of family 1 indicated. A λBstE II DNA molecular marker is shown in the first lane. Numerical indexing of the VNTR alleles is indicated on the right.

identifies variable number tandem repeat (VNTR) sequences (*21*). DNA from the eight members of family 1 was digested with Hinf I and, after DNA (Southern) blot transfer, was hybridized to the YNZ22.1 probe (*22*). Five polymorphisms for the YNZ22.1 allele were present in this family (Fig. 3). Alleles were numbered from 1 through 5 according to size. Five individuals whose constitutional DNA contains a mutant p53 gene all carry the YNZ22.1 allele No. 3 (Table 2). This demonstrates the co-segregation data confirm that co-segregation of this YNZ22.1 allele and the mutant gene in the family. The segregation data confirm members from three generations of family 1 have the identical germ line p53 gene mutation.

We next examined four additional LFS families (Fig. 1, families 2 through 5) for germ line p53 gene mutations. In family 2, lymphocytes and a portion of the breast cancer were available from member II-6. DNA encoding exons 5 through 8 of the p53 gene was first amplified from the lymphocytes. Mutation of a G to an A at the first nucleotide of codon 258 was found in three of four clones. This results in a switch from a glutamic acid to a lysine at this residue. (Table 2 and Fig. 2). This mutation apparently has not been noted as a polymorphism (*10, 11, 18*). All six clones containing DNA amplified from the breast cancer had the same mutation at codon 258 of the p53 gene. This suggests that a mutant p53 allele is present in this patient's constitutional cells, and that the breast cancer cells lost the remaining wild-type p53 allele. Because the DNA of this patient was uninformative for two p53 polymorphic markers and uninformative for the YNZ22.1 allele, it is difficult to confirm that the cells in the breast tumor had no remaining wild-type p53 protein (*20, 21*).

Osteosarcoma cells, normal tissue adjacent to the tumor, and skin fibroblasts were obtained from individual III-2 in family 3. Both the adjacent normal tissue and skin fibroblasts had a mutation of a C to a T at the first nucleotide of codon 245, which results in cysteine replacing the glycine at this position. The same mutation was found in three of four clones analyzed from the individual's osteosarcoma. In this case, the YNZ22.1 polymorphic probe was informative and showed a 70 percent reduction of one allele in the tumor sample. The partial loss of the wild-type allele is due to admixture of normal tissue in this sample (*20*). This suggests that individual III-2 in family 3 has a germ line p53 mutation at codon 245, and that cells within the tumor lost the remaining wild-type p53 allele.

Lymphocytes from individual III-3 in family 4 were analyzed for p53 gene mutations. Clones containing amplified DNA encoding p53 had the same C to T transition at the first nucleotide in codon 248 as found in family 1. The high frequency of codon 248 mutations in sporadic tumors and its presence in two of the five LFS families suggest a propensity to change at this site. Individual III-1 in family 5 has a germ line mutation at codon 252. In the first position of this codon there is a T to C change, which results in proline replacing leucine at this position.

All p53 germ line mutations noted in the LFS families are located in conserved region IV, where no polymorphisms have been detected and where nucleotide changes have previously been limited

Table 2. Analyses of p53 gene mutations in members of five families with Li-Fraumeni syndrome.

Patient	Tumor type	Age at diagnosis (years)	DNA source	Ratio mut/total clones	Codon	YNZ22.1 VNTR
Family 1						
Affected						
II.2	Breast	30	Fibroblasts	3/4	248	3,4
			Lymphocytes	4/6	248	
II.3	Bilateral breast	28	Fibroblasts	2/6	248	3,4
			Lymphocytes	3/6	248	
III.2	Brain	5	Fibroblasts	2/6	248	3,2
Carriers						
I.2			Fibroblasts	3/6	248	3,1
III.5			Fibroblasts	4/6	248	3,2
Non-carriers						
II.1			Fibroblasts	0/6		2,2
II.4			Fibroblasts	0/6		2,5
II.6			Fibroblasts	0/6		1,4
Family 2						
II.6	Breast	34	Lymphocytes	3/4	258	
			Tumor	6/6	258	
Family 3						
III.2	Osteosarcoma	19	Fibroblasts	2/6	245	1,2
			Normal	2/4		
			Tumor	3/4		2
Family 4						
III.3	Soft tissue sarcoma	5	Lymphocytes	2/4	248	
	Osteosarcoma	21	Lymphocytes	2/6*		
Family 5						
III.1	Soft tissue sarcoma	2	Fibroblasts	1/4	252	
	Osteosarcoma	14	Fibroblasts	1/4		
	Breast	31				

*In samples from family 4, patient III.3, separate PCR reactions were analyzed as indicated.

to tumor cells (*10, 11, 18*). All the mutations described in Table 2 were confirmed by a second PCR reaction and resequencing the gene products. Performing the PCR reactions in duplicate allowed us to overcome the inconsistencies due to the 0.25 percent rate of base misincorporation documented by others and previously confirmed in our laboratory (*10, 23*). Germ line p53 mutations were not identified in seven healthy controls and seven individuals with sporadic tumors who had no family history of cancer.

Possibility of restrictions on germ line p53 mutations. The half-lives of most mutant p53 proteins are markedly increased in comparison to the half-life of wild-type p53 protein. This is primarily a result of a complex they form with the heat shock proteins (hsc-70) (*24*). These mutant p53 proteins attain levels comparable to those known to exert a "trans-dominant loss of function" effect on wild-type p53 (*23, 25*). Other rare mutant p53 proteins that fail to form a complex with hsc-70 have a reduced capacity to override wild-type p53 function in transformation assays (*26*). We therefore asked whether such a trans-dominant loss of function effect of some mutant p53 proteins would restrict the types of germ line p53 mutations found in LFS families. The level of p53 protein was analyzed by immunoprecipitation of cell lysates from normal fibroblasts, LFS fibroblasts, and KHOS-240S cells (Fig. 4). The latter contain a p53 mutation at codon 156. This mutant protein forms a complex with hsc-70 (*27*). The high level of p53 protein found in the KHOS-240S cells is representative of the large amounts of protein found in cells with mutant p53 protein that complexes the hsc-70 proteins (*25*). In contrast, the p53 protein levels in the three LFS fibroblast lines are comparable to those of wild-type p53. The level of p53 protein in fibroblasts from individuals of 12 LFS families (including the four with documented germ line p53 mutations analyzed in Fig. 4), were also tested by immunofluorescence studies. All had low fluorescent intensities comparable to cells containing wild-type p53 protein (*20*). Both the immunoprecipitation and immunofluorescence studies probably indicate a selection against the p53 gene mutations that stabilize the mutant p53 protein that exerts a trans-dominant loss of function effect on the wild-type protein. Screening tests to determine germ line p53 mutations should not rely on methods that assume that the mutations will result in high levels of mutant protein.

Interpretations of the data. The presence of p53 mutations in the sporadic forms of many human tumors suggests that these alterations are an important step in the transformation of diverse cells. All cells in the individuals with LFS that we studied presumably have a single wild-type p53 allele. This finding provides an opportunity to compare the effects of p53 inactivation on the development of cancer in different tissues. The most remarkable feature of this syndrome is the diversity of tumors in the gene carriers. This suggests that numerous tissues are rendered more susceptible to transformation when they have only one wild-type p53 allele. The inactivation of p53 must therefore be at least one of the rate-limiting steps in the transformation of many tissues. However, tumors in LFS are not evenly distributed by site and do not follow cancer frequency patterns in the general population (Table 1). For example, breast carcinoma and colon carcinoma have comparable frequencies in the general population, and frequently have associated p53 mutations (*6*). In the LFS families, breast cancer occurs much more frequently than colon carcinoma (Table 1). The difference suggests that p53 inactivation is a more critical rate-limiting step in breast carcinoma formation than it is in colon carcinoma formation. Other patterns of tumor frequencies among affected individuals with LFS can provide additional clues regarding the differences in tissue-specific growth control pathways.

The development of molecular diagnostic tests to identify carriers of germ line p53 mutations provides a new tool for screening members of LFS families. Recently, an International Working Group on LFS was established, and nearly 100 candidate families have been enrolled. Members of these families can be examined for germ line p53 mutations. This effort should help clarify the spectrum of cancers attributable to germ line p53 gene alterations. In addition, gene carriers will be identified among the unaffected children and older relatives. A determination can also be made of the family members who are in fact, not carriers. The ability to detect germ line p53 mutations creates a responsibility to properly use this information, and to develop strategies for the care of gene carriers at exceptionally high risk of cancer.

The frequency of individuals in the general population who carry germ line p53 mutations is undefined. Li-Fraumeni syndrome might identify only the rare families in which germ line mutations in the p53 gene are highly penetrant and expressed in multiple tissues. The role of p53 germ line mutations in human cancer cannot be fully addressed by assessing its role in familial cancer. In retinoblastoma, for example, 85 percent of germ line mutation carriers, as identified by the presence of bilateral tumors, occur de novo (*28*). A germ line p53 mutation could occur in a substantial population of children and young adults who die of the cancer and do not propagate the gene. We are therefore seeking p53 mutations in individuals and families who display one or more features of LFS, such as multiple primary cancers or breast cancer in several young relatives. Those with a germ line p53 gene mutation are enrolled in a multicenter registry for further analyses.

In conclusion, we have shown that alterations of the p53 gene occur not only as somatic mutations in human cancers, but also as germ line mutations in some cancer-prone families. The frequency of germ line p53 mutations in cancer patients in the general population is unknown and merits further study. Both somatic and germ line mutations also arise in another tumor suppressor gene, the retinoblastoma gene. These finding indicate that the search for additional tumor suppressor genes can proceed along two parallel research

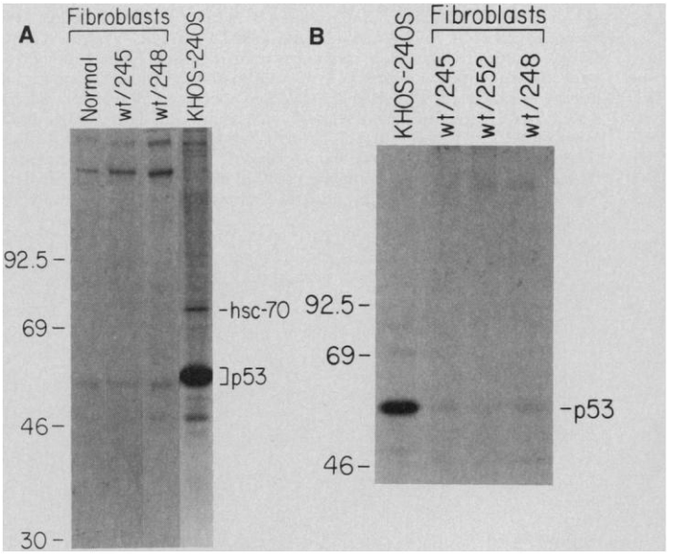

Fig. 4. Amount of p53 protein produced by various p53 alleles. Lysates from [^{35}S]methionine-labeled fibroblasts and KHOS-240S osteosarcoma cells were immunoprecipitated with monoclonal antibody pAb421 (*20, 30*). (**A**) (Lane 1) Normalfibroblasts; lane 2, wt/245 fibroblasts from (III-2) family 3 with a mutant codon 245 p53 allele; lane 3, wt/248 fibroblasts from (II-2) family 1 with a mutant codon 248 p53 allele; lane 4, KHOS-240S cells with a mutant codon 156 p53 allele. (**B**) (Lane 1) KHOS-240S cells as in Fig. 4A; (lane 2), the wt/245 mutant as in Fig. 4A; (lane 3) wt/252 fibroblasts from (III-1) family 5 with a mutant codon 252 p53 allele; (lane 4) wt/248 as in parens.

pathways. One is through analysis of the loss of heterozygosity in tumor samples, and the second is through the identification and study of cancer-prone families.

REFERENCES AND NOTES

1. F. P. Li, and J. F. Fraumeni, Jr., *Ann. Intern. Med.* **71**, 747 (1969).
2. _____, *J. Am. Med. Assoc.* **247**, 2692 (1982); J. E. Garber *et al., Proc Am. Assoc. Cancer Res.* **31**, 210 (1990).
3. J. M. Birch *et al., J. Clin. Oncol.* **8**, 583 (1990); J. M. Birch *et al., Br. J. Cancer* **49**, 325 (1984).
4. F. P. Li *et al., Cancer Res.* **48**, 5358 (1988).
5. W. R. Williams and L. C. Strong, *Familial Cancer, 1st International Research Conference* (Karger, Basel, 1985), p. 151; L. C. Strong *et al., J. Natl. Cancer Inst.* **79**, 1213 (1987).
6. J. L. Young, C. L. Perry, A. J. Asire, Eds., *Natl. Cancer Inst. Monogr.* **57**, KY72 (1981) (NIH publication no. **81**-2330).
7. V. M. Ricarrdi *et al., Pediatrics* **61**, 604 (1978); U. Francke, L. B. Holmes, L. Atkins, V. M. Riccardi, *Cytogenet. Cell Genet.* **24**, 185 (1979).
8. S. H. Friend *et al., Nature* **323**, 643 (1986).
9. C. A. Finlay, P. W. Hinds, A. J. Levine, *Cell* **57**, 1083 (1989); D. N. Eliyahu, D. Michalovitz, S. Eliyahu, O. Pinhasi-Kimhi, M. Oren, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8763 (1989); M. Isobe, B. S. Emanuel, D. Giron, M. Oren, C. Croce, *Nature* **320**, 84 (1986).
10. J. M. Nigro *et al., Nature* **342**, 705 (1989).
11. J. W. Romano *et al., Oncogene* **4**, 1483 (1989); L. M. Mulligan *et al. Proc. Natl. Acad. Sci.,* 5863 (1990); H. Ahuja, M. Bar-Eli, S. H. Advani, S. Benchimol, M. J. Cline, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6783 (1989); J. Bartek, R. Iggo, J. Gannon, D. P. Lane, *Oncogene* **5**, 893 (1990); R. Iggo, K. Gatter, J. Bartek, D. Lane, A. L. Harris, *Lancet* **335**, 675 (1990); S. J. Baker *et al., Science* **244**, 217 (1989); A. G. Menon *et al., Proc. Natl. Acad. Sci. U.S.A.* **87**, 5435 (1990); T. Takahashi *et al., Science* **246**, 491 (1989); B. Seizinger, unpublished results.
12. A. Lavigueur *et al., Mol. Cell Biol.* **9**, 3982 (1989).
13. All cell lines were maintained in Dulbecco's modified Eagle medium with glucose at 4.5 g/liter, L-glutamine, and sodium pyruvate. The medium was supplemented with 10 percent inactivated fetal calf serum and antibiotics. Cells were grown at 37°C in a humidified atmosphere containing 5 percent CO_2. Genomic DNA was extracted according to standard techniques (J. Sambrook *et al., Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, NY, ed. 2, 1989). To extract genomic DNA from blood lymphocytes, we first separated the cells from other blood components by passage through a Ficoll-Hypaque gradient. Tumor samples were processed after grossly contaminating normal tissue was removed. The tumor was then gently teased and homogenized into small fragments, which were resuspended in lysis buffer. The DNA was extracted from the resuspended tissue.
14. A 1.7-kb fragment was generated from 0.5 to 2.0 μg of the genomic DNA template. The sense and antisense primers used were, respectively. F1, 5′-ATGC GAATT CCCCT GCCCT CAACA AGAT-3′ starting at the first codon downstream to intron 4, and R2, 5′-TATAG GAATT CGTGG TGAGG CTCCC CTT-3′ starting at the ninth codon upstream of intron 8. Each primer had synthetic Eco RI sites at their 5′ ends to facilitate cloning. The DNA template and primers were added to a PCR buffer (DuPont) containing 50 mM tris-HCl (pH 9.5), 1.5 mM magnesium chloride, and 20 mM ammonium sulfate, to which were added dCTP, dATP, dTTP, and dGTP (at 0.2 mM each), and 0.5 U of Replinase (DuPont). The 100-μl reaction was amplified in a Perkin-Elmer Thermocycler (Cetus) for 35 cycles of 94°C (1 minute) 55°C (2 minutes), and 72°C (3 minutes). The reaction was initiated with one 80-second cycle at 94°C and ended with a 7-minute step at 72°C.
15. T. Soussi *et al., Oncogene* **5**, 945 (1990).
16. Ten reactions were performed for each DNA template, and the PCR products were pooled and digested with Eco RI, fractionated by electrophoresis, and after acid-phenol extraction and purification from low-melt agarose (New England Biolabs), fragments were ligated into a Bluescript vector "PBSK" (Stratagene) that had been digested with Eco RI and treated with calf alkaline phosphatase. After transformation, individual clones derived from standard minipreparations were sequenced in both directions with primers derived from the p53 coding and intron sequences. Analysis of six clones allows detection of both alleles more than 98 percent of the time. The use of either ^{35}S-labeled or ^{32}P-labeled nucleotides with T7 DNA polymerase yielded high autoradiographic resolution on a 12 percent polyacrylamide gel. The primer 5N, 5′-GGTTGGCTCTGACTGTA-3′, started at the first codon of exon 7 and generated the sequence of the region that contained the mutations described in the text.
17. V. L. Buchman, P. M. Chumakov, N. N. Ninkina, O. P. Samarina, G. P. Georgiev, *Gene* **70**, 245 (1988).
18. B. Vogelstein, unpublished results.
19. A. J. Levine, unpublished results.
20. S. H. Friend, unpublished results.
21. Y. Nakamura *et al., Nucleic Acids Res.* **16**, 5707 (1988); U. Masharani, D. Wolf, P. M. Frossard, *ibid.* **16**, 7757 (1988).
22. Southern blotting techniques were performed as described [J. Sambrook *et al., Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ed. 2, 1989), p. 9.31] for the capillary transfer method, Hybond nylon membranes (Amersham), and nonformamide containing hybridization solutions. The YNZ22.1 hybridization probe (*20*) was labeled by random priming the Klenow fragment of DNA polymerase, and [α-^{32}P]dCTP. Hybridization of the probe to 12 μg of genomic DNA digested with Hinf I was carried out at 65°C for 12 to 48 hours, and the blots were exposed to film for 3 to 15 days.
23. L. Diller *et al., Mol. Cell. Biol.* **10**, 5772 (1990).
24. H. W. Sturzbecher, P. Chumakov, W. J. Welch, J. R. Jenkins, *Oncogene* **1**, 201 (1987); P. W. Hinds, C. A. Finlay, A. B. Frey, A. J. Levine, *Mol. Cell. Biol.* 7, 2863 (1987); C. A. Finlay *et al., ibid.* **8**, 531 (1988).
25. P. W. Hinds, C. Finlay, A. J. Levine, *J. Virol.* **63**, 739 (1989).
26. P. W. Hinds *et al., Cell Growth Dev.*, in press.
27. J. W. Romano *et al., Oncogene* **4**, 1483 (1989).
28. J. Wiggs *et al., N. Engl. J. Med.* **318**, 151 (1988).
29. The presence of a guanine-guanine compression at codon 248 in the wild-type p53 gene is confirmed by sequencing both strands.
30. In preparation for immunoprecipitations, cell lines were metabolically labeled (at 50 μCi/ml) with methionine-free DME (ICN) in 2.5 ml of methionine-free DNA plus 2 percent dialyzed fetal calf serum for 2 hours. Cells were rinsed in ice-cold PBS twice before lysis in 1 ml of ELB (250 mM NaCl, 0.1 percent NP40, 50 mM Hepes, pH 7.0, 1 mM PMSF (Sigma), 5 mM EDTA, 0.5 mM DTT). The lysate was scraped from the plate, centrifuged, and stored at −80°C. Lysates were first adsorbed with fixed *Staphylococcus* protein A overnight at 4°C, then incubated with PAb122, before precipitation and loading on the 8 percent polyacrylamide gels.

31. We thank M. Dreyfus for data management; J. Garber, J. Schneider, and T. Russell for collecting specimens on families 1, 2, and 3; P. Begin and J. A. Cook for collecting specimens on families 4 and 5; W. W. Nichols for establishing cell lines on these two families; and S. E. Lux IV for important discussions, as well as S. Orkin and K. J. Isselbacher for critical review of the manuscript. Supported in part by the Baylor Fund Sub-Allocation for Research Program, Lucille P. Markey Foundation, and the Merck Foundation; a Research Fellowship of the Medical Research Council of Canada (D.M.); NIH grant 34936 (L.C.S. and M.A.T.); NIH grant 5-T32-CA09299 (F.Z.B); an Akvan T. and Viola D. Fuller American Cancer Society Fellow Junior Research Fellowship (D.H.K.); and a Lucille P. Markey Scholarship (S.H.F.).

24 September 1990; accepted 29 October 1990