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Germ-Line Mutations of *TP53* in Li-Fraumeni Families: An Extended Study of 39 Families[1]

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ABSTRACT

We have previously reported on the analysis of *TP53* coding mutations in 12 classic Li-Fraumeni syndrome (LFS) families plus 9 families that were Li-Fraumeni-like (LFL) families (J. M. Birch *et al.*, Cancer Res., *54:* 1298–1304, 1994). Mutations were found in 6 of 12 LFS families and in 1 of 9 LFL families. We have now extended these studies to include an additional nine LFS and nine LFL families, and *TP53* mutations have been detected in eight of nine LFS families and in three of nine LFL families. Six of the new mutations described here are the same as those previously identified in other Li-Fraumeni families and are missense mutations at codons 245, 248, and 273 (in two families); a nonsense mutation at codon 209; and a mutation at the splice donor site in exon 4. The other five mutations are novel germ-line mutations and include missense mutations at codons 136 and 344, a 2-bp deletion within codon 191, a splice acceptor mutation in intron 3, and a 167-bp deletion of part of exon 1 and intron 1. In addition, we have detected a codon 175 mutation in a family previously reported as *TP53* negative.

To summarize all of the data from the families we have studied in this and our previous report (J. M. Birch *et al.*, Cancer Res., *54:* 1298–1304, 1994), mutations have been detected in 15 of 21 LFS families (71%) and in 4 of 18 LFL families (22%). These figures are somewhat higher than those previously reported by us and others for the frequency of *TP53* mutations in LFS and LFL families. This could reflect our analysis of all 11 exons of *TP53*, including noncoding regions, as well as the use of direct sequencing rather than other less-sensitive mutation detection methods.

INTRODUCTION

In 1969, an inherited cancer predisposition syndrome was proposed by Li and Fraumeni on the basis of four families in which there were at least two cases of soft tissue sarcomas in early life. Other cancers noted at an increased frequency in these families were premenopausal breast cancer and other sarcomas (1), and the same group (2) subsequently defined the syndrome further as a proband with sarcoma diagnosed under the age of 45 years, with 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. In addition to sarcomas and premenopausal breast cancer, an excess of brain tumors, adrenal cortical tumors, and leukemias was noted (2). As more families have been identified, gastric cancer has also been reported at an increased frequency (3–5). Birch *et al.* (6) described additional families that did not precisely conform to the criteria of classic LFS[3] and that were termed LFL families. LFL families were defined on the basis of a proband with any childhood cancer or sarcoma, brain tumor or adrenal cortical tumor diagnosed under the age of 45 years with one first- or second-degree relative with a typical LFS cancer at any age, plus a first- or second-degree relative in the same lineage with any cancer under the age of 60 years.

In 1990, the underlying genetic lesion in some LFS families was identified as a mutation in the tumor suppressor gene *TP53* (7, 8), and there are now over 40 LFS and LFL families recorded with germ-line *TP53* mutations. However, around 50% of classic LFS families plus the majority of LFL families did not have a detectable coding mutation within *TP53*, and at least one LFL family has been excluded by linkage (9). It is probable that a proportion of these families does have a lesion within *TP53*, possibly due to mutations within introns or involving the promoter regions. A number of studies have analyzed groups of patients with tumors characteristic of LFS for germ-line *TP53* mutations. Such mutations have been identified in approximately 50% of patients with childhood adrenal cortical carcinoma (10, 11) and 10% of children with rhabdomyosarcoma (12) or brain tumors (13, 14). Thirty percent of children with sarcomas plus either multiple primary tumors (particularly those typical of LFS) or a family history have germ-line mutations (4–9, 15); however, adults with familial, early-onset, or bilateral breast cancer (16, 17) do not have germ-line *TP53* mutations to any significant degree (1–2%), although breast cancer is a principal component of LFS.

The types and position of germ-line *TP53* mutations largely seem to reflect those seen in sporadic tumors (18, 19), and many of the reported mutations are missense mutations within the conserved domains of *TP53*. Early reports indicated that mutations in LFS occurred in a tight cluster of codons within exon 7 (7, 8, 20), but subsequent reports from a number of laboratories have indicated that this clustering is not a general rule, although mutations at codons 245 and 248 are among the most frequent in LFS families, as they are in sporadic tumors (18, 19). As with all studies of mutations in *TP53*, however, it must be noted that the entire gene is not always analyzed, with many groups examining only exons 5–8 (7), and even some of the most comprehensive studies did not examine exon 1 (21). Whereas there is little doubt that mutations within conserved domains encoded by exons 5–8 are the most frequent in both sporadic tumors and in the germ line, the true frequency of mutations outside these domains remains to be determined.

We have ascertained 18 LFS or LFL families in addition to our previously published series. These families have been studied in detail for mutations to *TP53* by direct sequencing of all exons and splice junctions. Families that were negative in our previous study (6) were resequenced in this way. Additionally, all of the newly ascertained families together with all families recorded as negative for a *TP53* mutation in our previous report were examined for mutations within the *TP53* promoter region. In all families in which mutations have been detected, as many family members as possible have been analyzed, and the mutation has been confirmed. In the families described in an earlier report (6), additional family members have now been tested. This series, together with our previous report, forms the largest and most comprehensive series of LFS and LFL families studied to date.

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[3] The abbreviations used are: LFS, Li-Fraumeni syndrome; LFL, Li-Fraumeni-like.

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Table 1 *LFS and LFL families with germ-line TP53 mutations*

Family	Person	Relationship to proband	Tumor (age dx)[a] or current age if unaffected	Source DNA[b]	Exon[c]	Codon	Nucleotide change	Amino acid change	Restriction digest[d]
Classic LFS families									
16	IV-2	Proband	RMS (1)	Fixed (t)	6	220	TAT→TGT	Tyr→Cys	
	IV-1	Sister	ACC (1)	Fixed (t)	6	220	TAT→TGT	Tyr→Cys	
	III-5	Mother	B (27)	Fixed (t)	6	220	TAT→TGT	Tyr→Cys	
	II-2	Maternal aunt	CIN (31)	Fixed (t)	NMF				
	III-2	Mother's cousin	ST (23)	Fixed (t)	NMF				
83	II-3	Brother	LPS (39) NF (49)	Blood	5	175	CGC→CAC	Arg→His	*Hha*I (−)
	II-2	Proband	FS (3) Os (10, 13)	Fixed (t)	5	175	CGC→CAC	Arg→His	*Hha*I (−)
	III-2	Nephew	RMS (2)	Fixed (t)	5	175	CGC→CAC	Arg→His	*Hha*I (−)
	III-3	Nephew	ALL (15)	Blood	5	175	CGC→CAC	Arg→His	*Hha*I (−)
	III-4	Nephew	Unaffected (20)	Blood	5	175	CGC→CAC	Arg→His	*Hha*I (−)
	I-1	Father	Unaffected (77)	Blood	NMF				
	I-2	Mother	Unaffected (77)	Blood	NMF				
	II-4	Brother's wife	Unaffected (44)	Blood	NMF				
84	IV-2	Proband	Med (3) Os (8)	Fixed (t)	7	248	CGG→CAG	Arg→Gln	*Hpa*II (−)
	III-4	Maternal aunt	B (25, 44) LMS (44)	Blood	7	248	CGG→CAG	Arg→Gln	*Hpa*II (−)
	IV-3	Sister	Unaffected (22)	Blood	NMF				
86	IV-5	Mother	Unaffected (36)	Blood	Intron 3		Acceptor ag→aa		
	V-3	Proband	Os (15)	Blood	Intron 3		Acceptor ag→aa		
	III-1	Maternal grandfather's cousin	Ov (58)	Blood	NMF				
	III-2	Maternal grandfather's cousin	C (43)	Blood	NMF				
110	III-8	Mother	B (32) Sch (33)	Fixed (t)	7	248	CGG→CAG	Arg→Gln	*Hpa*II (−)
	IV-7	Proband	Os (29)	Fixed (t)	7	248	CGG→CAG	Arg→Gln	*Hpa*II (−)
	III-5	Maternal aunt	Gl (33)	Fixed (t, n)	NMF				
117[e]	III-2	Mother's cousin	Gl (34)	Fixed (t)	5	175	CGC→CAC	Arg→His	*Hha*I (−)
	III-6	Mother's cousin	G (35)	Blood	5	175	CGC→CAC	Arg→His	*Hha*I (−)
	III-8	Mother	Ab (29) B (32)	Fixed (t)	5	175	CGC→CAC	Arg→His	*Hha*I (−)
	III-13	Maternal aunt	As (31)	Blood	5	175	CGC→CAC	Arg→His	*Hha*I (−)
	IV-1	Mother's 2nd cousin	Unaffected (14)	Blood	5	175	CGC→CAC	Arg→His	*Hha*I (−)
	IV-2	Mother's 2nd cousin	ACC (2)	Fixed (t)	5	175	CGC→CAC	Arg→His	*Hha*I (−)
	III-1	Mother's cousin's wife	Unaffected (nk)	Blood	NMF				
	III-5	Mother's cousin	Unaffected (29)	Blood	NMF				
	III-11	Maternal uncle	Unaffected (31)	Blood	NMF				
	III-15	Maternal aunt	Unaffected (43)	Blood	NMF				
	IV-14	Cousin	CIN II/III (19)	Blood	NMF				
222	III-1	Proband	RMS (3)	Blood	7	248	CGG→CAG	Arg→Gln	*Hpa*II (−)
	III-2	Brother	Os (14)	Fixed (t)	7	248	CGG→CAG	Arg→Gln	*Hpa*II (−)
	II-2	Mother	B (33)	Fixed (t)	7	248	CGG→CAG	Arg→Gln	*Hpa*II (−)
	II-1	Father	Unaffected (36)	Blood	NMF				
	I-2	Maternal grandmother	?L (71)	Blood	NMF				
	II-3	Mother's half-sister	Unaffected (49)	Blood	NMF				
	II-4	Mother's half-sister	Unaffected (49)	Blood	NMF				
	III-3	Sister	Unaffected (9)	Blood	NMF				
266	II-2	Sister	ACC (1) B (29)	Blood	7	248	CGG→TGG	Arg→Trp	*Hpa*II (−)
	II-4	Proband	RMS (3) Ch (14) MM (15)	Blood	7	248	CGG→TGG	Arg→Trp	*Hpa*II (−)
	I-2	Mother	B (33)	Fixed (t)	7	248	CGG→TGG	Arg→Trp	*Hpa*II (−)
	I-1	Father	Unaffected (nk)	Blood	NMF				
267	III-2	Proband	ACC (1) RMS (15)	Blood	4	108–111	Deletion-insertion	Gly-Phe-Arg-Leu→Ile-Gln	
	II-2	Mother	BB (40)	Blood	4	108–111	Deletion-insertion	Gly-Phe-Arg-Leu→Ile-Gln	
	III-3	Brother	Unaffected (17)	Blood	4	108–111	Deletion-insertion	Gly-Phe-Arg-Leu→Ile-Gln	
	III-4	Brother	Unaffected (9)	Blood	4	108–111	Deletion-insertion	Gly-Phe-Arg-Leu→Ile-Gln	
	II-1	Father	Unaffected (44)	Blood	NMF				
610	II-1	Father	Os (26) L (27)	Blood	5	136	CAA→TAA	Gln→Stop	
	III-2	Proband	RMS (3)	Blood	5	136	CAA→TAA	Gln→Stop	
	I-2	Paternal grandmother	B (39)	Blood	NMF				
1786	III-2	Mother	B (31) LPS (41)	Blood	1	Del 167 bp	—	—	
	IV-2	Proband	LPS (14)	Blood	1	Del 167 bp	—	—	
2180	III-3	Proband	RMS (10)	Blood	8	273	CGT→TGT	Arg→Cys	
7003	III-6	Proband	Os (33) LMS (44)	Blood	10	344	CTG→CCG	Leu→Pro	*Alu*I (−)
	III-9	Brother	Os (<40)	Blood	10	344	CTG→CCG	Leu→Pro	*Alu*I (−)
2635	III-2	Sister	B (48), Skin (?)	Blood	4	Splice donor	ACG→ACA	(Thr→Thr)	
	IV-1	Niece	BB (39)	Blood	4	Splice donor	ACG→ACA	(Thr→Thr)	
	IV-7	Niece	Mes (40)	Blood	4	Splice donor	ACG→ACA	(Thr→Thr)	
	II-3	Mother	Unaffected (90)	Blood	NMF				
2612	III-1	Father	T (32) SpS (34)	Blood	6	209	AGA→TGA	Arg→Stop	
	IV-1	Proband	NHL (16)	Fixed (t)	6	209	AGA→TGA	Arg→Stop	
LFL families									
85	IV-1	Proband	ACC (1)	Blood	5	180	GAG→AAG	Glu→Lys	*Nla*III (−)
	III-7	Father	Unaffected (52)	Blood	5	180	GAG→AAG	Glu→Lys	*Nla*III (−)
	V-1	Son	Unaffected (6)	Blood	NMF				
	III-6	Father's cousin	B (43)	Fixed (t)	NMF				
	III-12	Paternal uncle	Bl (43)	Fixed (t, n)	NMF				
1502	III-3	Half-sister	B (29)	Blood	8	273	CGT→CAT	Arg→His	*Nla*III (+)
	III-1	Proband	B (28), As (28)	Blood	8	273	CGT→CAT	Arg→His	*Nla*III (+)
2252	III-1	Maternal grandmother	B (26, 40) LMS (49)	Blood	6	191 (del 2 bp)	TCCTCA→TTCA	—	*Dde*I (−)
	IV-1	Mother	DCIS (25)	Blood	6	191 (del 2 bp)	TCCTCA→TTCA	—	*Dde*I (−)
	IV-2	Maternal uncle	Lip (20s)	Blood	6	191 (del 2 bp)	TCCTCA→TTCA	—	*Dde*I (−)
	V-1	Proband	As (11)	Blood	6	191 (del 2 bp)	TCCTCA→TTCA	—	*Dde*I (−)

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Table 1 *Continued*

Family	Person	Relationship to proband	Tumor (age dx)[a] or current age if unaffected	Source DNA[b]	Exon[c]	Codon	Nucleotide change	Amino acid change	Restriction digest[d]
	II-2	Mother's great uncle	R (55)	Fixed (n, t)	NMF				
	III-2	Mother's maternal aunt	FB (43) M (45)	Fixed (t)	NMF				
	IV-3	Maternal aunt	Unaffected (29)	Blood	NMF				
7391	II-1	Proband	B (34)	Blood	7	245	GGC→AGC	Gly→Ser	*AciI* (−)
	II-4	Brother	As (54)	Blood	7	245	GGC→AGC	Gly→Ser	*AciI* (−)

[a] Age dx, age in years at diagnosis. Tumor types are abbreviated as follows: Ab, astroblastoma; ACC, adrenal cortical tumor; ALL, acute lymphoblastic leukemia; As, astrocytoma; B, breast; BB, bilateral breast; Bl, bladder; C, cervical carcinoma; Ch, chondrosarcoma; CIN, cervical intraepithelial neoplasia; DCIS, ductal carcinoma *in situ* of the breast; FB, fibrocystic breast disease; FS, fibrosarcoma; G, gastric; Gl, glioma; L, lung; Lip, lipoma; LPS, liposarcoma; LMS, leiomyosarcoma; M, mesenchymoma; Med, medulloblastoma; Mes, mesothelioma; MM, malignant melanoma; NF, neurofibroma; NHL, non-Hodgkin's lymphoma; Os, osteosarcoma; Ov, ovarian; R, rectum; RMS, rhabdomyosarcoma; Sch, schwannoma; SpS, spindle cell sarcoma; ST, seminoma testis; T, teratoma; nk, age at diagnosis or last follow-up not known.

[b] DNA was purified from blood or fixed tissue. The latter was either of normal material or tumor blocks and is indicated by n and t, respectively. The percentage of tumor cells in the blocks varied and is discussed further in the text as appropriate.

[c] NMF, no mutation found.

[d] The loss or gain of a recognition site for the enzymes shown are indicated by − and +, respectively. No entry in this column indicates that no diagnostic digest was possible.

[e] Previously reported as negative for a *TP53* mutation, see Varley *et al.* (3) for more details.

MATERIALS AND METHODS

Ascertainment of the Families. Families were incorporated in this study if they met the criteria for LFS (2) or LFL (6) families. The families were ascertained through the Manchester Children's Tumour Registry, the United Kingdom Children's Cancer Study Group, and the Family History Clinics at the Christie and Withington Hospitals (Manchester, United Kingdom) and through sarcoma patients attending the Cancer Research Campaign Department of Medical Oncology at the Christie Hospital. In addition, a small number of families were ascertained by direct referral to one of the authors. Details of tumors in family members are given in Tables 1 and 2, together with the relationship to the proband in each family.

Molecular Studies. Blood samples were obtained from the majority of patients; where it was not possible to obtain fresh material, DNA was extracted from paraffin-blocked pathology material as described previously (6). Initial screening for *TP53* mutations involved the analysis of one affected family member, optimally using DNA isolated from peripheral lymphocytes. Any suspicious finding was confirmed by analyzing DNA from a second affected family member plus others, if such material was available. Individuals tested are shown in Tables 1 and 2. All mutations were verified by either sequencing the complementary strand or diagnostic restriction digest (see Table 1). In families in which a mutation was detected, at least two family members carried the same mutation. In only one family were we unable to analyze *TP53* in a second affected individual (family 2180).

All *TP53* exons were sequenced in all families. However, in those families where a mutation was detected before all exons had been screened, if this mutation was verified as described above, no additional exons were sequenced. In practice, exons 4–7 were generally screened first, and if no mutation was detected, all of the other exons were examined. Details of the primers and PCR conditions are available from the authors. Sequencing was carried out using either an ABI 373 or 377 sequencer using both dye primer and dye terminator chemistries. All families reported as negative in the original study (6) have been entirely resequenced using automated methods, which are much more sensitive than the manual sequencing methods originally used by us.

Linkage Analysis. Two polymorphic repeat sequences were analyzed for segregation within families in which there were sufficient family members to warrant analysis. The repeat sequences studied were a dinucleotide $(CA)_n$ repeat (22) and a pentanucleotide $(AAAAT)_n$ repeat within the first intron of *TP53* (23). Families 117 and 81 were the only families in which linkage analysis was appropriate; other families had either been shown to carry a germ-line *TP53* mutation or were unsuitable for analysis because of insufficient material from affected family members. Primary and secondary PCRs were carried out, and the products were separated on denaturing acrylamide gels as described previously (24).

RESULTS

Nine LFS and nine LFL families were screened for germ-line *TP53* mutations by direct sequencing of all exons and splice junctions. Initial analysis was carried out on one affected member of each family, but in all families, the presence of the mutation was confirmed in at least one other affected individual (Table 1), except for family 2180. In all families, blood from at least one individual was tested, except in family 110, in which only fixed paraffin-embedded material was available from three affected individuals.

Mutations were detected in eight LFS and three LFL families (Table 1). In six families, the mutations were identical to those described previously in LFS families (families 110, 2180, 2612, 2635, 7391, and 1502). In the other families, the mutations had not been previously identified as germ-line mutations. In addition, a family that had been reported in our earlier study (6) as *TP53* negative was found to have a codon 175 mutation (CGC→CAC, Arg→His) and is included in Table 1. This family represents one of the most extensive LFS families studied and has been reported in detail (3). The person numbers given in Table 1 are those given in Ref. 3. This family had been previously analyzed by the HOT (hydroxylamine, osmium tetroxide) technique on DNA from fixed tumor material from IV-2, which failed to detect the mutation. Linkage analysis on this family indicated that a mutation within or close to *TP53* could be the causative defect (3). Direct sequencing of DNA obtained from blood from two affected individuals (III-8 and III-13) clearly showed a codon 175 mutation, and reanalysis of the tumor DNA from IV-2 showed that there was an almost complete loss of the wild-type allele (3).

Linkage analysis in the second family large enough to study did not rule out *TP53* as the causative mutation. However, only six individuals could be studied, and although *TP53* could not be excluded, the number of individuals informative for the two markers studied was too small for formal linkage analysis.

A codon 248 mutation was detected in family 110, which brings to four the number of families in our total series with a mutation within this codon. The amino acid change in this family (Arg→Gln, CGG→CAG) is the most common seen at this residue in all reports of germ-line mutations and has been found in 3 of 4 families in our total series. Of particular interest in this family is the observation that individual III-5 does not carry the codon 248 mutation. III-5 presented with a glioma at 33 years, and we obtained paraffin-embedded material from the tumor and adjacent normal tissue. The material was reviewed by one of us (A. M. K.), and the sections used to isolate DNA were assessed as >80% tumor cells and 100% normal cells, respectively. The codon 248 mutation was not detected in either sample. Sections were recut from the blocks, reviewed, and retested with the same result. III-5 had a son with rhabdomyosarcoma at <1 year, and other branches of the family have tumors consistent with LFS. III-5's son died in 1955, and we have been unable to obtain

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Table 2 *Cancers in probands and their first- and second-degree relatives in LFS and LFL families in which no germ-line TP53 mutation has been found*

Family	Person	Relationship to Proband[a]	Tumor (age at diagnosis)[b]
Classic LFS families			
22	III-5	Proband[c]	Os (2) T-NHL (9)
	III-4	Sister[c]	T-NHL (6) GI (14)
	III-3	Brother	Leuk (3)
	I-2	Paternal grandmother	R (70)
81	IV-5	Proband	RMS (2)
	IV-2	Sister	B (41)
	IV-3	Sister	B (37)
	IV-4	Brother	Benign histiocytoma cutis (32)
	III-2	Paternal uncle	L (73)
	III-4	Father	Colon (58)
	III-5	Mother[c]	BB (44, 45)
82	IV-3	Proband	RMS (4)
	IV-2	Sister	Congenital hemangiopericytoma (0)
	IV-5	Brother[c]	ALL (14)
	II-2	Maternal grandmother	C (48)
88	II-2	Proband[c]	FS (25)
	I-2	Mother	CIS C (34) BB (36)
	II-5	Half-sister	B (29)
119	III-2	Proband	Ch (34)
	III-1	Brother[c]	ST (33)
	II-1	Paternal aunt	LyS (21)
1799	III-7	Proband[c]	STS (37) B (37)
	II-7	Father	G (48)
	II-6	Paternal uncle	Bo (50s)
	II-5	Paternal uncle	Li (40)
	II-4	Paternal uncle	G (42)
	I-1	Paternal grandfather	Cancer NOS (?)
LFL families			
69	IV-2	Proband	Triton (5)
	IV-1	Sister[c]	Wilms' tumor (0)
	III-5	Mother	CIN (33)
	III-1	Paternal uncle	P (37)
	III-2	Paternal uncle	Retroperitoneal tumor (31)
	II-5	Paternal grandmother	C (52)
80	V-4	Proband[c]	LPS (30) B (35)
	IV-19	Mother	RMS (52) R (66)
	IV-17	Maternal aunt	R (45)
	III-19	Maternal grandmother	B (66)
253	IV-2	Proband[c]	ALL (5)
	III-2	Mother	B (32)
	II-2	Maternal grandmother	Bo (50)
328	III-2	Proband	Os (14)
	II-3	Mother[c]	B (45)
	II-4	Maternal uncle	GI (56)
	II-6	Maternal uncle	L (64)
338	IV-3	Proband	PNET (7)
	III-2	Mother[c]	Ch/Os (35) Lip (37) B (38)
348	III-2	Proband[c]	Synovial sarcoma (27)
	II-3	Father	Hep (61)
	II-1	Mother	BB (45, 54) E (57) Ov (58)
	I-1	Maternal grandmother	B (73) SCC (80)
352	IV-1	Proband	ALL (1)
	III-3	Mother[c]	B (28)
	III-1	Maternal aunt	CIN III (27)
	II-3	Maternal grandmother	L (49)
353	III-3	Proband[c]	S (0)
	III-2	Brother	Co (27)
	II-2	Father	G (55)
729	IV-2	Proband[c]	Os (9)
	III-2	Mother[c]	B (42)
	II-2	Maternal grandmother	B (52)
1761	IV-2	Proband[c]	B (22)
	IV-1	Brother	Leuk (22)
	III-1	Father	P (32)
	III-3	Paternal aunt	B (32)
	III-6	Paternal aunt	Leuk (3)
	III-7	Paternal aunt	B (32)
	II-1	Paternal grandmother	B (29)
2063	III-1	Sister[c]	BB (47, 49)
	II-2	Mother	BCC (48) B (58)
	II-4	Maternal uncle	? Cancer of spine
	I-2	Maternal grandmother	? Stomach
2093	IV-3	Proband[c]	Os (16)
	III-1	Father	Unknown primary (49)
	III-2	Mother	L (55)
	II-4	Maternal aunt	B (45)
2613	III-1	Proband[c]	PNET (16)
	III-2	Brother[c]	As (8)
	II-1	Father	L (46)
2634	III-3	Proband[c]	B (29)
	III-2	Brother	G (36)
	II-7	Father	G (44)
	II-8	Mother	G (59)

[a] In some cases, the proband is unaffected and is therefore not shown on this table.

[b] Abbreviations for tumor types are as defined in Table 1, but in addition, the following abbreviations are used in this table: BCC, basal cell carcinoma; Bo, bowel; Cancer NOS, cancer not otherwise specified; CIS C, carcinoma *in situ* of the cervix; Co, colon; E, esophagus; Hep, hepatoma; Leuk, leukemia not otherwise specified; Li, liver; LyS, lymphosarcoma; P, pancreas; PNET, primitive neuroectodermal tumor; S, sarcoma; SCC, squamous cell carcinoma; STS, soft tissue sarcoma; T-NHL, T-cell non-Hodgkin's lymphoma; Triton, Triton tumor.

[c] Individuals tested for *TP53* mutations.

material for analysis. We have not been able to sequence the other exons because of the very limited amount of material available to us, and we have therefore been unable to exclude the possibility that a second germ-line mutation is segregating within family 110.

A nonsense mutation at codon 136 (CAA→TAA, Gln→Stop) was detected in family 610. The presence of the mutation was confirmed in two family members: (*a*) a man with osteosarcoma of the jaw at 26 years and adenocarcinoma of the lung at 27 years; and (*b*) his daughter, diagnosed with alveolar rhabdomyosarcoma at 3 years. The same mutation was not detected in I-2, who was diagnosed with breast cancer at 39 years, and whose father was reported to have died of stomach cancer at an unknown age. There are two possibilities for this negative finding. Either there was a new mutation within this family with the breast cancer in I-2 being coincidental, or the mutation is inherited through I-1, who is unaffected but not available for study. There are no reports of codon 136 germ-line mutations and only 17 reports of mutations to codon 136 in sporadic tumors (19), 10 of which are nonsense mutations of Gln→Stop, with 2 others being an insertion and a deletion that would result in frameshift mutations with premature termination.

Individual III-3 in family 2180 was shown to have a mutation at codon 273 (CGT→TGT, Arg→Cys). We obtained pathology material from her affected sister and mother, but we were unable to amplify *TP53* sequences from this material. Another relative was diagnosed with gastric cancer at 50 years, but we were unable to obtain material from this individual. Although we have been unable to confirm the presence of the mutation in other family members, this mutation has been described in other LFS families (21), and so it seems likely that it is the causative defect.

An additional codon 273 mutation (CGT→CAT, Arg→His) was detected in LFL family 1502. This mutation differed from that in family 2180 but has also been described as a germ-line mutation (25, 26). The same mutation was present in two half-sisters whose father died of carcinoma of the pancreas at 37 years. A variety of germ-line codon 273 mutations have been described including Arg→Gly (27) and Arg→Ser (28) as well as those discussed above.

Two individuals from LFL family 7391 (II-1 and II-4) had an identical codon 245 mutation (GCC→AGC, Gly→Ser). Codon 245 mutations have also been described previously in the germ line, including the identical mutation as seen in family 7391 (4, 16, 29), as well as Gly→Cys (7) and Gly→Asp (30). Family 7391 is a LFL family and is characterized by several brain tumors of unknown type and two early-onset breast cancers (at 34 and 21 years; see Table 1). As yet, no childhood tumors or sarcomas have been identified or diagnosed in this family. There is one other report in the literature of a family with predominantly brain tumors (31), and two studies have identified frequent germ-line *TP53* mutations in patients with brain tumors, particularly those with early-onset tumors, multiple primaries, or an unusual family history (13, 14).

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The third LFL family in which a *TP53* germ-line mutation was detected was family 2252, with a deletion of 2 bp within codon 191 (CCCCTCCTC→CCCCT–TC, Fig. 1) leading to premature termination at the equivalent of codon 207, with an altered COOH terminus from codon 191-ter. A truncated and presumably inactivated product is predicted, lacking a number of key domains including part of the DNA-binding domain and the tetramerization domain. The same mutation was detected in III-1 and IV-1, with loss of the wild-type allele in all tumors studied (32). II-1 died of gastric cancer at 23 years; her brother (II-2) died of cancer of the rectum at 55 years. Tumor and normal tissue from II-2 were examined, and no mutation was found at codon 191; this patient therefore represents a phenocopy within this family. III-2 was also examined because she had presented with a number of benign lesions including fibrocystic breast disease and a benign mesenchymoma, but she did not carry the mutation. IV-2 and IV-3 were also tested; IV-2 carried the mutation, whereas IV-3 did not. Both were asymptomatic at the time of testing. There are only six reports in the database (19) of mutations involving codon 191. Three of these reports describe missense mutations of Pro→Thr/His/Ser (33–35), but the other three describe deletions of between 1 and 10 bp (Refs. 36–38; see Fig. 1). The deletion reported here occurs within a pyrimidine tract, and both this deletion and those reported by others (36, 38) can be explained by slippage during DNA replication by simple misalignment. The larger 10-bp deletion described (37) is flanked by a 2-bp repeat (CT; see Fig. 1), and two reviews (39, 40) indicated that any multiple-bp deletions tended to involve direct repeats of 2–8 bp. A model of slipped mispairing during replication can account for such deletions (41) and has been described in a number of germ-line mutations in a variety of genes. Although the codon 191 *TP53* deletion in family 2252 is a germ-line deletion, and the others discussed above are somatic mutations, the mechanisms of generation of the deletion will be similar.

A novel mutation at codon 344 was detected in two affected individuals of family 7003. The mutation is the first described at this codon in either the germ line or in sporadic tumors (19) and is a Leu→Pro (CTG→CCG) change within the tetramerization domain. Mutations within the tetramerization domain have not been described in the germ line and have only rarely been reported in sporadic tumors, with the majority of these being nonsense mutations. The crystal structure of the tetramerization domain has been described (42), and Leu^{344} is a key residue within the α-helical domain. A Leu→Pro missense mutation at this position is predicted to disrupt oligomerization, and the monomeric mutant p53 protein may lose the ability to bind to DNA in a sequence-specific manner. Studies in which residues in the COOH terminus of *TP53* were randomly mutated indicated that a Pro^{344} mutant had lost the ability to transactivate target genes and to arrest in the G_1 phase of the cell cycle after DNA damage and also showed partial cell growth inhibition (43). However, data from studies of mouse *TP53* indicate that loss of the tetramerization domain may not result in loss of the ability of the mutant p53 to transactivate target genes or loss of its tumor suppressor properties (44). It has been suggested that retention of these two key activities by p53 proteins mutant within the tetramerization domain is the reason why they are rarely selected in tumors (19, 42, 44). The identification of a germ-line mutation within this domain and loss of the wild-type allele in a tumor from one patient (45) indicate that mutations within this domain may be of more relevance than previously thought. In support of this, a second Li-Fraumeni family has recently been described in which there is a mutation within the tetramerization domain (46). Given that exon 10 is frequently not screened in these families, germ-line mutations within the tetramerization domain may be relatively common. Family 7003 is somewhat unusual in that there are no confirmed childhood tumors, although the spectrum of tumors is typical of LFS. It is possible that the relatively late onset of tumors in the family reflects the nature of the mutation.

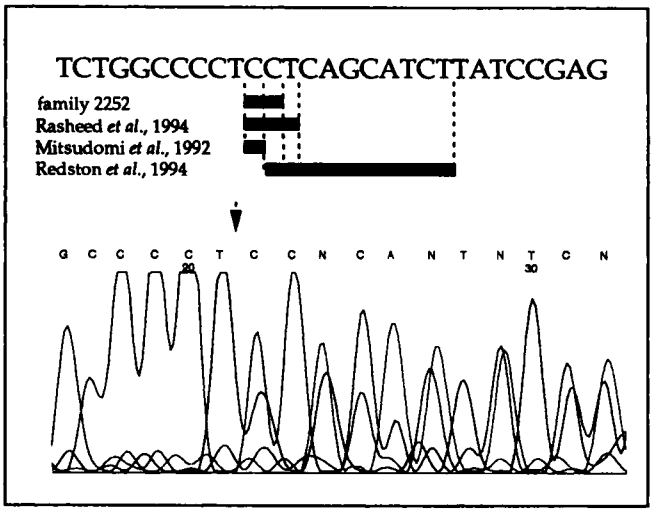

Fig. 1. *Upper panel*, the sequence of *TP53* around codon 191 showing the deletion in family 2252 and a comparison with deletions in the same region in sporadic tumors. *Boxes*, deleted nucleotides. *Lower panel*, an electropherogram of DNA from individual IV-1 showing the position of the deletion (*arrow*).

A splice acceptor mutation was detected in two members of family 86. The mutation affects the absolutely conserved nucleotide at the splice junction (ag/TC→aa/TC) and is therefore predicted to perturb correct splicing. There are four other reports in the literature of germ-line *TP53* mutations that affect splicing (21, 47–49); however, none are at the same position.

A second LFS family (family 2635) was found to have a splice mutation, affecting the splice donor site at exon 4 (CG/gt→CA/gt). An identical mutation has been reported for another LFS family, and in this study, the authors verified that splicing of intron 4 did not occur, leading to the expression of a larger *TP53* transcript than normal (48). It is possible that the family reported here and that reported by Warneford *et al.* (48) are related, particularly because there is only one report of an identical mutation in the database of somatic mutations (19).

A nonsense mutation in codon 209 was detected in two affected family members of family 2612 (AGA→TGA, Arg→stop). There is one other report of an identical germ-line mutation (28) in a child with an osteosarcoma but no unusual family history of cancer, and there are also a number of identical mutations in sporadic tumors (19).

The final family in which a germ-line *TP53* mutation was detected is family 1786, in which a deletion of 167 bp was shown to have removed part of exon 1 and intron 1 (Fig. 2). Amplification by PCR of exon 1 using primers 5756 and 93147 in III-2 and IV-1 produced a fragment of the expected size (674 bp) plus an additional smaller product of approximately 500 bp. The smaller product seemed to be more abundant than the normal product, possibly due to the number of amplification cycles carried out (35). Sequencing the products using primer 93147 (see Fig. 2) gave a readable sequence with a high background. The sequence was as expected until nucleotide 1051 (numbering according to P. M. Chumakov; GenBank accession number X54156), when there was divergence from the expected sequence (Fig. 2), consistent with a 167-bp deletion between nucleotides 884 and 1051. Sequencing the same PCR product with internal primer 93079 gave the readable sequence of the normal exon 1, again with a high background, presumably due to the presence of the interfering abundant smaller PCR product. The binding site for primer 94570 has been removed by the deletion in the mutant *TP53* allele, therefore only

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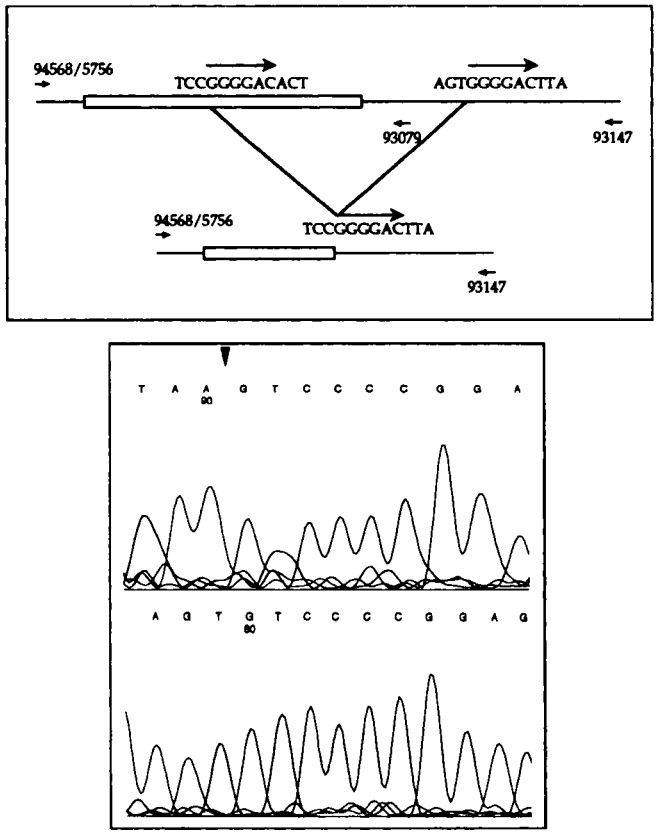

Fig. 2. *A*, diagrammatic representation of the deletion in family 1786 within exon 1 and intron 1. The primers used for amplification of exon 1 are indicated. The *upper part* of the diagram shows the wild-type allele, the *lower part* shows the germ-line deletion event in individuals III-2 and IV-1. *Arrows*, the 6-bp repeat flanking the deleted region. *B*, electropherograms showing the sequence of the mutant allele obtained with primer 93417 (*top*) and the sequence of the wild-type allele obtained with primer 93079 (*bottom*). *Arrow*, the deletion breakpoint. Note that the reverse sequence is shown.

the wild-type allele is sequenced. Analysis of the sequence at the junction of the deletion shows the presence of a 6-bp sequence that is repeated at nucleotides 885–890 and 1052–1057 (GGGGAC; see Fig. 2). This repeat bears striking similarity to a consensus repeat identified (41) as that flanking hot spots for deletions in a number of genes ($TG^{G}/_{A}{}^{G}/_{A}{}^{G}/_{T}{}^{A}/_{C}$). This sequence is also similar to the immunoglobulin switch region consensus sequence, TGGGG.

The deletion in family 1786 removes 65 bp of exon 1 including the splice donor site plus 102 bp of intron 1. The likely consequence of this deletion is that there is aberrant splicing of a cryptic splice site within intron 1 to exon 2. Although the promoter region of human *TP53* has not been fully characterized for regulatory elements, those that have been identified are not removed by the deletion. It is therefore possible that the mutant allele is transcribed. The initiation codon is within the second exon, therefore translation may be possible, but it seems likely that transcription, splicing, or translation is perturbed. Studies are under way to investigate this further.

DISCUSSION

In the present report and our previous study (6), we have analyzed a total of 39 Li-Fraumeni families. Fifteen of 21 classic LFS families (71%) and 4 of 18 LFL families (22%) were found to have germ-line *TP53* mutations. These figures are higher than those reported by other groups and may reflect the method we have used to detect mutations. All families have been examined by direct sequencing of all exons plus the promoter region including 209 bp upstream of the major transcription start site (position 522; numbering according to P. M. Chumakov; GenBank accession number X54156). Most other groups have analyzed only the hot-spot exons (exons 5–8), using a variety of techniques including constant denaturant gel electrophoresis (CDGE), denaturing gradient gel electrophoresis (DGGE), single-strand conformational polymorphism, or the HOT technique, as well as direct sequencing. The former techniques may fail to detect a proportion of mutations. Functional assays have been developed that can distinguish mutations based on the ability of wild-type p53 to transactivate target genes (50, 51), but they will not detect large deletions, promoter mutations, or mutations in which the mutant allele is poorly expressed in the tissue studied (50). Of the 19 mutations that we have identified in our Li-Fraumeni families, 5 (26%) are outside the hot-spot regions: (*a*) family 267 with a complex deletion-insertion at codons 108–111 in exon 4; (*b*) family 1786 with a deletion involving part of exon 1 and intron 1; (*c*) family 86 with a splice acceptor mutation in intron 3; (*d*) family 2635 with a splice donor mutation in exon 4; and (*e*) family 7003 with a missense mutation in exon 10. This indicates the need to sequence the entire gene in Li-Fraumeni families.

We have seen a relatively high frequency of germ-line *TP53* mutations within LFL families (22%) in our complete series of families. The criteria we apply to define LFL families are more stringent than those used by other groups, with some families only marginally failing to meet the criteria for classic LFS. Eeles (52) defines LFL families as the occurrence of two different tumors typical of LFS in individuals of any age who are first- or second-degree relatives with respect to each other and reports a mutation frequency of only 7% in 61 families studied. It seems, therefore, that our more stringent definition of LFL may explain the higher frequency of mutation detected in our series of families, with families conforming to the looser definition (52) including some linked to other genetic defects as well as a proportion of chance clusters.

Of the 19 families we have identified with germ-line *TP53* mutations, we have only seen 1 family in which we have a proven *de novo* mutation. Both parents in family 83 were tested, and neither has the codon 175 mutation that is present in their twin sons. We cannot rule out a *de novo* mutation in family 610, in which individual I-2 was diagnosed with early-onset breast cancer (at 39 years) but is wild-type. We have not been able to test her husband, who is unaffected but whose father died of cancer of the bowel at 49 years, and it is therefore possible that the mutation is inherited through the paternal side of the family. Initial testing of I-2 was carried out on DNA from lymphocytes, but we subsequently received paraffin-embedded material from her breast tumor, normal breast tissue, a benign uterine fibroid, and normal tissue surrounding the fibroid. No codon 136 mutation was seen in any tissue, and if I-2 was a gonadal mosaic, her breast tissue might be expected to carry the mutation.

In a number of families, there are cancer-affected family members who do not carry the germ-line *TP53* mutation present in other individuals within the same family. The most surprising of these is in family 110, in which III-5 does not carry the codon 248 mutation present in her sister and nephew, in spite of having a typical Li-Fraumeni tumor herself (a glioma at 33 years) and a son who died of a rhabdomyosarcoma at <1 year. Examination of the extended pedigree (data not shown) shows that there are a number of affected individuals on the maternal side, including early-onset breast cancer and retroperitoneal soft tissue sarcoma. Although the likelihood of a second germ-line mutation within 1 family seems extremely small, there is 1 report of a patient with 2 independent germ-line mutations (14) of only approximately 100 independent mutations in the literature. We have been unable to fully analyze the DNA for the presence of a second mutation due to lack of material, and a mutation in the other allele remains a possibility.

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In family 16, a second-degree relative of the affected mother of the proband (6) with carcinoma *in situ* of the cervix at 31 years and her son with a seminoma of the testis at 23 years did not have the codon 220 mutation segregating in this family. Although neither of these tumors is characteristic of Li-Fraumeni families, their occurrence in a family such as this might be considered suspicious. In an additional family (family 85; Ref. 6), two affected individuals with breast cancer at 43 years and bladder cancer also at 43 years were wild-type at codon 180. The male affected individual with bladder cancer is the brother of a mutation carrier, whereas the female affected relative with breast cancer is a cousin, and her parents are both unaffected. In family 86, two maternal grandfather's cousins were affected with ovarian and cervical cancer at 58 and 43 years, respectively, but both were wild-type for the mutation segregating within this family. The final family in which there are affected individuals who do not carry the germ-line mutation segregating within the family is in family 2252. This family is described in full in Varley *et al.* (32). Cancer is a very common disease, and within any extended pedigree, a number of sporadic cancers would be expected. However, the number of relatively early-onset tumors observed in Li-Fraumeni family members who do not carry the germ-line *TP53* mutation present in that family may indicate that there are other factors involved, such as modifier genes. A large collaborative multicenter study is required to address this. It should be pointed out, however, that we have not always been able to sequence the entire *TP53* gene in individuals who do not carry the germ-line mutation present in other members of the same family because of the limited amounts of material available in many cases. Nonetheless, it would be prudent to confirm the mutation in tissue from a first-degree relative before offering the mutation as a presymptomatic test to unaffected relatives.

Tumors in which germ-line *TP53* mutations are known to be involved include bone and soft tissue sarcomas, brain tumors, adrenal cortical tumors, leukemias, and early-onset breast tumors (2, 53). Melanoma and gonadal germ cell tumors have also been suggested to be part of the syndrome (53–55). We (3) and others have suggested that gastric tumors may be a component tumor. From the families reported here, together with our earlier series (6), it seems that other tumors may also be more frequent in Li-Fraumeni families. Tumors of the pancreas are present in five families in our total series (family 1761, at 32 years; family 80, at 56 years; family 7003, at 49 years; family 1502, at 37 years; and family 69, at 37 years). Carcinoma of the esophagus has also been seen in four Li-Fraumeni families (family 84, at 50 years; family 7003, at 27 years; family 338, at 53 years; and family 348, at 57 years).

It can be seen from Table 1 that a number of unaffected individuals have been examined for the presence of *TP53* mutations. In particular, a number of children have been studied. All family members have given their consent to be included in the research studies reported in this paper, as have the parent(s) of any children studied. It has been made clear to the families that they are participating in a research project and that they will not be given the results of this research. They are, however, informed that if at any time results become available that mean a predictive genetic test could be devised for their family, they will be given the option to enter a formal genetic counseling and predictive testing program. Any such testing will be carried out on duplicate clinical samples in a Service Diagnostic Laboratory. Issues relating to predictive presymptomatic testing are more fully discussed in Ref. 56.

In view of our findings that germ-line *TP53* mutations can be found throughout the entire gene, including both coding and noncoding exons, in Li-Fraumeni families, we consider it essential to analyze the entire gene when screening such families. Any mutation that is detected must be verified by sequencing the complementary strand and, if possible, by restriction digest analysis. Mutations that have not been described previously should be confirmed as mutations and not polymorphisms by screening a large number of normal alleles (>100). In addition, candidate mutations should be analyzed wherever possible using one of the available functional assays. It should be noted in particular that our finding of cancer-affected family members who are not mutation carriers has important implications for predictive testing and assessment in LFS and LFL families with germ-line *TP53* mutations. As many individuals as possible should be screened for the presence of the mutation within a family, and in particular, if presymptomatic testing is to be offered within families, confirmation of the mutation in a first-degree relative is highly desirable. Only if this sort of screening is carried out can we obtain reliable data on penetrance and possible genotype/phenotype correlations.

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