Prevalence and Diversity of Constitutional Mutations in the p53 Gene among 21 Li-Fraumeni Families


ABSTRACT

The entire coding sequence of the p53 gene was analysed for the presence of mutations in 12 families conforming to a restricted definition of Li-Fraumeni syndrome (classic LFS) and nine families with features of LFS conforming to a broader definition. Mutations were detected in seven families. Six were point mutations with one each affecting codons 175, 180, and 220 and three affecting codon 248. The seventh was a deletion/insertion mutation in exon 4. Germline mutations in p53 were a feature of six of the nine families with such tumors. An analysis of these 7 mutations, together with 34 published examples, showed that more than one-half were transitions at CpG dinucleotides, suggesting that the majority of germline p53 mutations may arise as a result of spontaneous events. The most common cancers occurring in the 41 families with germline p53 mutations, in common with classic LFS, were bone and soft tissue sarcoma, breast cancer, brain tumors, leukemia, and adrenocortical carcinoma, although less than one-half of the probands with germline p53 mutations came from classic LFS families. More than one-half of the cancers overall and nearly one-third of the breast cancers were diagnosed before 30 years of age. These observations have important implications for asymptomatic carriers of germline p53 mutations, and there is a need for international collaboration in the development of protocols for the management of such families.

INTRODUCTION

LFS was initially defined on the basis of the history of cancer in four families in which siblings or cousins were affected with soft tissue sarcoma during infancy or childhood. The close relatives of these children had a high incidence of premenopausal breast cancer, sarcomas, and other cancers occurring at unusually early ages. Li and Fraumeni suggested that these findings represented an inherited cancer family syndrome (1).

Systematic analyses of cancer incidence in the families of population-based or hospital-based series of children with bone and soft tissue sarcoma have subsequently supported the notion of genetic susceptibility to a diverse but specific spectrum of cancers in certain families (2–5).

In order to study the characteristics of the syndrome, Li et al. (6) assembled data on 24 kindreds conforming to a defined set of criteria. Eligible families included one individual, the proband, diagnosed with sarcoma before 45 years of age, a first degree relative with cancer before 45 years of age, and another first or second degree relative in the lineage with any cancer diagnosed during this age interval, or sarcoma occurring at any age. This definition of LFS has been widely applied and accepted, and families conforming to this definition are hereafter referred to as "classic" LFS.

Brain tumors, leukemia, and adrenocortical carcinoma, diagnosed under age 45 years, occurred to excess among the 24 families in addition to bone and soft tissue sarcomas and breast cancer. The systematic studies referred to above, together with a recent follow-up study of the 24 families (7), confirm these cancers as the principal components of the syndrome and in addition suggest that other cancers including melanoma, gonadal germ cell tumors, and possibly other embryonal tumors of childhood may also be associated with the syndrome.

For many years, the genetic basis of LFS remained elusive, but two years ago the occurrence of germline mutations in the p53 tumor suppressor gene in 6 families with LFS was reported (8, 9). In sporadic tumors, including the principal component syndrome cancers, p53 mutations are frequently found. These mutations have been observed throughout the gene but are concentrated within four evolutionarily conserved domains (10, 11). The 6 germline mutations initially reported all occurred within a stretch of 14 codons in exon 7 in conserved region 4. We subsequently published the results of an analysis of exon 7 in eight classic LFS families. Only two of the families had germline mutations in exon 7 (12).

Since then, there have been a number of reports of germline mutations in individual families (13–17) and among a small minority of patients with sarcomas (18–20), second malignant neoplasms (21, 22), childhood leukemia (23), and breast cancer (24–26). In total, these reports include 36 individuals or families with germline p53 mutations. The family histories were consistent with the definition of classic LFS in less than one-half of these.

The observed mutations occurred in exons 4–9, involved nonconserved as well as conserved residues, and included a splicing mutation and nonsense as well as missense mutations. Thus, the spectrum of mutations and the phenotype of patients and families with germline p53 mutations is broader than was suggested by the initial reports.

In the majority of the above studies, only the conserved exons of the p53 gene were analysed. Furthermore, in general these reports lack details of families investigated for the presence of p53 mutations in the germline but in whom no such mutations were detected. The purpose of the present study was to determine the spectrum of mutations and the proportion of LFS families with germline p53 mutations.

We have therefore analyzed the entire coding sequence in a series of 21 systematically ascertained LFS families. The series includes the eight families previously analyzed for the presence of mutations in exon 7 only (12) plus 13 additional families.
**Materials and Methods**

**Ascertainment of Families.** Twelve families conforming to the definition of classic LFS (6), and nine families with features suggesting LFS but not strictly conforming to the definition were included in the study. The latter families, hereafter referred to as “LFS-like families,” were included if they met the following criteria: proband with any childhood cancer or sarcoma, brain tumor, or adenocortical carcinoma diagnosed under 45 years of age, with one first or second degree relative with typical LFS cancer (sarcoma, breast cancer, brain tumor, leukemia, or adenocortical carcinoma) diagnosed at age 60, plus one first or second degree relative in the same lineage with any cancer diagnosed under age 60. This broader definition is based on the characteristics of classic families including estimates of age-specific cancer risk which is elevated up to but not beyond 60 years of age (3, 7).

Among the 12 classic families, 5 were ascertained through systematic studies of the families of children included in the Manchester Children’s Tumour Registry (Fig. 1, families 16 and 81–84). Five families were identified through the United Kingdom Children’s Cancer Study Group patient register (families 22, 117, 222, 266, and 267), and 2 families were identified through a systematic survey of family history in patients with sarcomas attending the CRC Department of Medical Oncology in Manchester (families 88 and 119).

Among the LFS-like families, 7 were ascertained through the Manchester Children’s Tumour Registry (Fig. 2, families 85, 253, 328, 338, 352, 353, and 729), and 2 were identified through the Department of Medical Oncology (families 80 and 348).

Nuclear families only, including those cancer-affected members on whom the classification of classic LFS or LF-like was based, are shown in the pedigrees. In most of these families, cancers had also occurred in members of other generations and branches.

**Analysis of p53.** DNA was extracted from blood and from fixed, paraffin-embedded tissue according to standard procedures (27, 28).

In families 16, 80–85, 88, 119, 222, 266, 267, 328, 338, 348, 352, and 353, exons 1–10 and the translated region of exon 11 were analyzed by direct sequencing. For exons 5 to 8, we used the primers and conditions described by Hsu et al. (29). The amplification primers were made in both 5’ biotinylated and unbiotinylated forms. The other exons were amplified using a biotinylated and an unbiotinylated primer. The unbiotinylated primer was used subsequently for sequencing. The primers used were: exon 1, bioCTGAGGACAAACGCAAAGCTTTCGCT and ~GAGTCCGATGTGCCTATCACATCC; exon 2, bioAAGCTCTCAGCTGATTCCC and CAGGAAAGCTCTGAGGAC; exon 3, GGAACTGTGAGTGGAGAAGCCA; exon 4, bioAATTGATGCTGCTATTCCAGAGTACC; and AACCTTCCACCTGAAAGGCTC; exon 9, bioGAACCCAGGTACATGCTGCAGG; exon 9, bioGAACCCAGGTACATGCTGCAGG; exon 9, bioGAACCCAGGTACATGCTGCAGG; and bioGAACCCAGGTACATGCTGCAGG.

**Cytochrome C (CYC) Mutations.** Constitutional cytochrome C mutational screening for patients with germline p53 mutations in children with sarcoma was undertaken on blood lymphocytes, lymphoblastoid cell lines, or cultured skin fibroblasts from eight classic LFS and seven LFS-like families.

**Results**

<table>
<thead>
<tr>
<th>Family</th>
<th>Person</th>
<th>Source DNA</th>
<th>Exon</th>
<th>Codon</th>
<th>p53 Mutations</th>
<th>Base change</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic LFS families</td>
<td>016</td>
<td>003</td>
<td>Fixed tissue</td>
<td>6</td>
<td>220</td>
<td>TA → TGT</td>
<td>Tyr → Cys</td>
</tr>
<tr>
<td></td>
<td></td>
<td>011</td>
<td>Fixed tissue</td>
<td>6</td>
<td>220</td>
<td>TGT → TGT</td>
<td>Tyr → Cys</td>
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<td></td>
<td>083</td>
<td>002</td>
<td>Blood</td>
<td>5</td>
<td>175</td>
<td>CGC → CAC</td>
<td>Arg → His</td>
</tr>
<tr>
<td></td>
<td></td>
<td>004</td>
<td>Blood</td>
<td>5</td>
<td>175</td>
<td>CGC → CAC</td>
<td>Arg → His</td>
</tr>
<tr>
<td></td>
<td>003</td>
<td>Blood</td>
<td>No mutation found</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>084</td>
<td>001</td>
<td>Fixed tissue</td>
<td>7</td>
<td>248</td>
<td>CGG → CAG</td>
<td>Arg → Gin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>035</td>
<td>Blood</td>
<td>7</td>
<td>248</td>
<td>CGG → CAG</td>
<td>Arg → Gin</td>
</tr>
<tr>
<td></td>
<td>222</td>
<td>001</td>
<td>Blood</td>
<td>7</td>
<td>248</td>
<td>CGG → CAG</td>
<td>Arg → Gin</td>
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<td>7</td>
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<td>Blood</td>
<td>No mutation found</td>
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<tr>
<td></td>
<td>266</td>
<td>004</td>
<td>Blood</td>
<td>7</td>
<td>248</td>
<td>CGG → TGG</td>
<td>Arg → Trp</td>
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<td></td>
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<td>006</td>
<td>Fixed tissue</td>
<td>7</td>
<td>248</td>
<td>CGG → TGG</td>
<td>Arg → Trp</td>
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<tr>
<td></td>
<td>267</td>
<td>002</td>
<td>Blood</td>
<td>4</td>
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<td>GGT TGC GGT CTG</td>
<td>Gly Phe Arg Leu</td>
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<tr>
<td></td>
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<td>006</td>
<td>Blood</td>
<td>4</td>
<td>108→111</td>
<td>GGT TGC GGT CTG</td>
<td>Gly Phe Arg Leu</td>
</tr>
<tr>
<td></td>
<td>005</td>
<td>Blood</td>
<td>No mutation found</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LF-like family</td>
<td>085</td>
<td>001</td>
<td>Blood</td>
<td>5</td>
<td>180</td>
<td>CAG → AAG</td>
<td>Glu → Lys</td>
</tr>
<tr>
<td></td>
<td></td>
<td>002</td>
<td>Blood</td>
<td>5</td>
<td>180</td>
<td>CAG → AAG</td>
<td>Glu → Lys</td>
</tr>
</tbody>
</table>
mutation in the germline. Details of individuals tested and mutations found are given in Table 1. For completeness, the 2 mutations reported previously in families 84 and 266 are included in Table 1. Since the families represent a consecutively ascertained series, exclusion of these two families would distort the results. In Fig. 1 and 2, * indicates individuals in whom constitutional DNA was analyzed for the presence of p53 mutations. The person numbers indicated on the pedigrees cross-reference with Table 1 to show the results of the p53 analyses in these individuals. Fig. 3 shows autoradiographs of the sequencing gels indicating mutations at the first and second nucleotides of codon 248 in families 266 and 84, respectively. An additional family (222) also carried a mutation affecting the second nucleotide of codon 248. Three of the remaining germline mutations detected in 2 classic LFS families and 1 LFS-like family were also single base changes affecting codons 175 (family 83), 220 (family 16), and 180 (family 85), respectively. Five of these mutations occurred within the conserved domains of the p53 gene. The mutation affecting codon 220 was outside the conserved regions but affected a conserved residue. The final mutation in exon 4 (family 267) was also outside the conserved regions of the gene and was a complex mutation involving deletion of 11 base pairs and insertion of 5 base pairs. This mutation did not, however, alter the reading frame. Fig. 4 shows an example of the chemical cleavage of mismatch technique: hydroxylamine modification of segment II in five individuals. The p53 gene was amplified in eight segments to generously incorporate all intron/exon boundaries. Segment II (nucleotide 11918 to nucleotide 12446; HSP53G; EMBL accession number XL54156) incorporated exon 4 and covered the 16-base pair insertion polymorphism of intron 3 (32), the codon 72 polymorphism (33), and a C->A polymorphism at nucleotide 11933 in intron 3.4 Except in track 3, the distinguishing bands are variously due to these changes. Track 3 shows additional bands due to the complex deletion/insertion mutation of family 267 in this study. Constitutional cytogenetic studies on at least 1 affected member from 15 of the families did

Fig. 1. Twelve families conforming to the definition of classic LFS (6). ACC, adrenocortical carcinoma; ACT, adrenocortical tumor; ALL, acute lymphoblastic leukemia; B, breast carcinoma; BB, bilateral breast carcinoma; b.HC, benign histocy toma; C, colon carcinoma; CNS, central nervous system tumor; CS, chondrosarcoma; FBS, fibrosarcoma; GS, gastric sarcoma; GO, glioma; HPC, hemangiopericytoma; LMS, leiomyosarcoma; LPS, liposarcoma; MB, medulloblastoma; MM, malignant melanoma; NHL, non-Hodgkin lymphoma; OS, osteosarcoma; RMS, rhabdomyosarcoma; SM, seminoma of testis; SNS, synovial sarcoma; square, male; black square, male with cancer; shaded square, male with benign tumor; circle, female; black circle, female with cancer; diagonal line, individual is dead; arrows, proband; *, individual tested for presence of p53 mutation.

Unpublished result.
p53 MUTATIONS IN LI-FRAUMENI FAMILIES

Fig. 2. Nine LF-like families conforming to a broader syndrome definition (see "Materials and Methods"). ACC, adrenocortical carcinoma; ACT, adrenocortical tumor; ALL, acute lymphoblastic leukemia; B, breast carcinoma; BB, bilateral breast carcinoma; BL, bladder carcinoma; C, colon carcinoma; CR, colorectal carcinoma; CS, chondrosarcoma; CUK, metastatic carcinoma of unknown primary site; CX, cervical carcinoma; E, esophageal carcinoma; G, gastric sarcoma; GO, glioma; L, lung carcinoma; LPS, liposarcoma; O, ovarian carcinoma; OS, osteosarcoma; PNET, primitive neuroectodermal tumor; R, rectal carcinoma; RMS, rhabdomyosarcoma; SCC, squamous cell carcinoma; SNS, synovial sarcoma; square, male; black square, male with cancer; shaded square, male with benign tumor; circle, female; black circle, female with cancer; diagonal line, individual is dead; arrows, proband; *, individual tested for presence of p53 mutation.

Fig. 3. Mutations detected at codon 248 in families 84 and 266.

not reveal any chromosome abnormalities (classic families 16, 22, 81, 84, 88, 117, 222, and 267; LFS-like families 80, 85, 253, 328, 338, 348, and 353).

DISCUSSION

In this survey of 21 families with features of LFS, 7 were identified as carrying germline mutations in the p53 gene. Six of these were among the 12 families fulfilling the definition for classic LFS and include the 2 mutations in exon 7 reported previously by us (12). Only one such mutation was detected in the nine LFS-like families, conforming to the wider definition. It therefore seems that the criteria for classic LFS define a group of families at higher risk of carrying constitutional p53 mutations than the broader definition.

It is of interest that among the classic families, five of the six with germline p53 mutations included young children with rhabdomyosarcoma. Three of these families and the only LFS-like family with a germline p53 mutation included infants with adrenal cortical carcinoma. None of the families in which probands had adult-onset sarcomas or childhood soft-tissue sarcomas other than rhabdomyosarcoma carried germline p53 mutations. It would seem, therefore, that in addition to the criteria for classic LFS, the presence of rhabdomyosarcoma or adrenal cortical carcinoma in young children may also point to a high risk of carrying p53 mutations in the germline. Two families, 22 and 82, fulfill the definition for classic LFS having probands with childhood sarcoma each with 2 affected siblings. Unusually for classic LFS families, the parental generation is not affected. Furthermore, in neither family was a p53 mutation detected, and the involvement of a recessive gene inherited from each parent is perhaps suggested.

Mutations in codon 248 occurred in 3 families in our series. Codon 248 was the site of mutation in 2 of the families originally reported (7), and 3 other examples of germline mutations in this codon have subsequently been published (17, 19, 20). It therefore appears that codon 248, which is a mutational "hot spot" in sporadic tumors (34), is additionally a "hot spot" for germline mutations. Codon 175 is also a mutational "hot spot" in sporadic tumors, but the present report represents the first example of a constitutional mutation in this codon. Following the initial discovery of p53 mutations in the germline and the positional clustering of these mutations between codons 245 and 258, there was some speculation as to whether only certain mutations were permitted in the germline with others being lethal (7, 17, 35). The allele mutant for codon 175 is 3- to 10-fold more efficient than those for certain other "hot spot" residues in transformation assays (10) and conferred the ability to grow in soft-agar when introduced into SAOS-2 cells (36). Furthermore, the mutant protein has the ability to complex hsc 70, whereas most other mutants found in the germline do not (37). The occurrence of this mutation in the germline may therefore indicate that there are no restrictions on the types of germline missense mutations which can occur.

Only one example of the mutation in codon 220 found in family 16 is recorded in a sporadic tumor in the review by Caron de Fromental and Soussi (34). In family 16, the mutation was found in two affected first degree relatives of the proband and is therefore likely to be significant. The mutation at codon 180 has not previously been recorded, but as this occurs within a conserved domain, and was inherited by the proband from her father, whose family has a strong history of cancers occurring at early ages, it is likely to be biologically significant.

The deletion/insertion mutation occurring in exon 4 is unusual, and no germline mutations of this complexity have previously been re-
porting. Toguchida et al. (18) have reported germline mutations in nonconserved domains involving the insertion of one base pair (two examples) or deletion of two base pairs (one example), but each of these three mutations resulted in a frameshift leading to premature stop codons. The present mutation did not lead to an alteration of the reading frame, but since it was detected in the proband and his affected mother and was not present in his unaffected father, it is probable that this mutation does account for the high incidence of cancer in this family. Further work on the biological significance of these mutations and the properties of their protein products is in progress.

In this study, we have analyzed the entire coding sequence of p53 and were able to detect constitutional mutations in only 6 of the 12 families with classic LFS. Clearly, the genetic basis of cancer predisposition cannot be accounted for by such mutations in all LFS families. The basis of the high incidence of cancer in the remaining families is not known, but the inactivation of normal p53 function by other mechanisms cannot be excluded.

Most investigators have analyzed only the conserved regions in exons 5 through 8. If these exons alone are considered, it will be seen that, including the present study, 16 of the 41 documented mutations occur in exon 7, with 8 affecting codon 248 and 4 affecting codon 245. An additional 12 mutations occurred in exon 8, with codons 273 and 282 each affected 4 times. Only four examples of mutations outside of exons 5 through 8 have been documented. However, these are likely to be underrepresented, as most investigators have not analyzed the nonconserved regions. The distribution of these mutations is represented in Fig. 5.

Thirty of the 41 documented constitutional mutations were transitions, and 21 of these occurred at CpG dinucleotides. There were five examples of transversions and five deletion and/or insertion mutations. It is interesting to note that, although carcinoma of the breast is one of the most common cancers to occur in LFS families, in sporadic breast cancers, transversions are as common as transitions; mutations at CpG sites are relatively infrequent. The distribution of mutational types in the constitutional examples more closely resembles that of sporadic colon cancers than sporadic breast cancers. The hypermutability of CpG sites through the spontaneous deamination of 5-methylcytosine is well documented (34), and the frequency of this type of mutation in the germline suggests that the majority arise as a result of spontaneous events rather than exposure to exogenous mutagens.

The detection of germline p53 mutations presents difficult clinical and ethical problems with respect to the management of families with such mutations (38, 39). For example, in relation to asymptomatic carriers, age-, sex- and site-specific cancer risks associated with constitutional p53 mutations are unknown. The families and individuals in whom such mutations have been detected represent a highly selected group investigated largely because of striking histories of cancer. However, some general observations regarding types of cancers and ages at diagnosis can be made.

Only 16, fewer than one-half of the 41 germline p53 mutations occurred in classic LFS families, 9 occurred in LFS-like families, 11 in patients with multiple primary cancers, 3 in breast cancer patients with family histories of breast and other cancers not consistent with LFS, and 2 in sarcoma patients with 1 cancer-affected first degree relative. Table 2 compares the characteristics of cancers occurring in these “p53 families” with those in the generality of classic LFS families. The latter are based on data derived from systematic studies of LFS and from unselected series of children with sarcoma (3, 6, 7, 40). From this comparison, it can be seen that the two groups are remarkably similar. It would seem reasonable, until substantial specific data on cancer risks in individuals with p53 germline mutations are available, to use the data derived from general studies of LFS and from unselected series of children with sarcoma (3, 6, 7, 40) as a basis for counseling and planning screening strategies in carriers of such mutations.

The very young age at onset of breast cancers in carriers of germ-line p53 mutations, with over 30% occurring under the age of 30 and virtually all cases occurring before the age of 50, should be particularly noted. Given the reported inefficiency of mammography in detecting breast cancers in younger women, together with a predicted susceptibility to radiogenic cancers (41), there may be a real need to develop alternative screening procedures for breast cancer in carriers of germ-line p53 mutations.

Coordinated multidisciplinary protocols for the management of families with p53 germline mutations are being initiated in the United

Table 2. Comparison of characteristics in LFS families and all families with germline p53 mutations

<table>
<thead>
<tr>
<th>Classic LFS families</th>
<th>p53 families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most common cancers</td>
<td>Most common cancers</td>
</tr>
<tr>
<td>Bone and soft tissue sarcoma</td>
<td>Bone and soft tissue sarcoma</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>Brain tumor</td>
<td>Brain tumor</td>
</tr>
<tr>
<td>Leukemia</td>
<td>Leukemia</td>
</tr>
<tr>
<td>Adrenocortical carcinoma</td>
<td>Adrenocortical carcinoma</td>
</tr>
<tr>
<td>Multiple primary cancers common</td>
<td>Prone to develop multiple primary cancers</td>
</tr>
<tr>
<td>50% cancers diagnosed before 30 years</td>
<td>63% cancers diagnosed before 30 years</td>
</tr>
<tr>
<td>28% breast cancers diagnosed before age 30 years and 89% before age 50 years</td>
<td>32% breast cancers diagnosed before age 30 years and 97% before age 50 years</td>
</tr>
<tr>
<td>No excess cancers above population rates beyond age 59 years</td>
<td>Only 4 of 158 cancers in known or assumed carriers of germline p53 mutations occurred after age 59 years</td>
</tr>
</tbody>
</table>

* Derived from this report and Refs. 3, 6, 7, and 32.

† Derived from this report and Refs. 3, 6, 7, and 32.

ACKNOWLEDGMENTS

The expert technical assistance of Catherine Rackstraw is gratefully acknowledged. We are also grateful to members of the United Kingdom Children’s Cancer Study Group for their support and to histopathologists throughout the United Kingdom who provided material. We also thank consultants, general practitioners, medical records departments, and cancer registries staff who provided confirmatory diagnostic information.

REFERENCES


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