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Li–Fraumeni Syndrome

J.M. Birch

In 1969, F.P. Li and J.F.Fraumeni described four families in which childhood soft tissue sarcoma was associated with early onset breast cancer and other early onset cancers in their close relatives. Three of these families were identified through pairs of siblings with rhabdomyosarcoma occurring among a series of nearly 650 children with rhabdomyosarcoma in the U.S.A. By making assumptions about family size, Li and Fraumeni estimated that considerably less than one pair of affected siblings would have been expected by chance among this series of children. A further family in which a pair of cousins were affected was also identified. On investigation of the family histories of these four pairs of children, Li and Fraumeni found an unusually high incidence of breast cancer occurring premenopausally in close female relatives, sarcomas also occurring at an early age, and other unusually early onset cancers, including acute leukaemia in other close family members. It was of particular interest that three of the mothers of the index children with soft tissue sarcoma had developed breast cancer under the age of 30 years. Li and Fraumeni proposed that the observed clustering of cancers in these families was due to inherited predisposition [1].

In a subsequent more detailed report, a second pair of cousins with soft tissue sarcoma occurring in childhood was identified, and adrenocortical carcinoma and brain tumours were observed in first degree relatives of other children with soft tissue sarcoma included in the series. These latter observations suggested that these cancers may also be components of the proposed cancer predisposition syndrome [2].

A family showing a similar pattern of cancers had been previously reported [3], and following Li and Fraumeni's reports, other families with patterns of cancer also consistent with their findings were described [4–6]. In a report describing a series of families based on breast cancer probands, in which other unusual clustering of cancers consistent with Li and Fraumeni's findings was observed, Lynch and colleagues coined the term "SBLA syndrome". These letters representing what these authors regarded as the component syndrome cancers, that is, sarcoma (S), breast and brain (B), leukaemia lung and larynx (L) and adrenocortical carcinoma (A) [7]. This term has been used by others, but the syndrome was also commonly known as the Li–Fraumeni syndrome [6], and Li–Fraumeni syndrome (LFS) is now the accepted term.

It was, however, uncertain whether these familial clusters were due to a genetically determined predisposition to a broad but specific range of cancers. There was a prevailing concept at this time that inherited predisposition to cancer could occur only in a site-specific fashion. The acceptance of the notion that a single trait could result in predisposition to such a diverse spectrum of neoplasms represented a problem. Alternative explanations were that exposure to common environmental agents within families may have generated these clusters of cancers, or such clusters may simply represent rare chance aggregations in certain families. Strong support for the notion of inherited susceptibility to component cancers in families with LFS was subsequently provided by systematic studies of families and patient populations. A number of pieces of evidence indicating genetic predisposition emerged from these studies.

The first piece of evidence came from a follow-up study

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conducted by Li and Fraumeni in their original four families. They found that, over a 12-year period, 10 of the 31 surviving family members had developed 16 additional cancers in comparison with less than one expected from U.S. population rates. These 16 cancers were of the same types as had originally been observed in the families, and included five breast cancers, four soft tissue sarcomas and two central nervous system tumours. Although the soft tissue sarcomas had all occurred within the fields of previous radiotherapy, when these were excluded the remaining number of cancers still represented a highly significant excess above expectation, with 12 cancers occurring compared with 0.5 expected. The second piece of evidence also emerged from this follow-up study. It was noted that 12 of the cancers occurred in individuals who had survived an original cancer. These 12 cancers which occurred in 8 patients were mainly sarcomas and carcinoma of the breast. This high frequency of second and subsequent cancers of types originally described among the families further supported the idea of genetic predisposition to these cancers in the affected individuals [8].

Definitive evidence came from studies of cancer incidence in the families of a population-based series of children with soft tissue sarcoma and a hospital-based series of survivors of childhood soft tissue sarcoma reported by groups in the United Kingdom and the United States, respectively [9–12]. The first population-based survey of familial cancer among children with soft tissue sarcoma was conducted by Birch and colleagues [9]. This study was based on an analysis of cancer incidence in the mothers of children included in the Manchester Children's Tumour Registry with a diagnosis of soft tissue sarcoma. Results demonstrated an increased incidence of cancer in these mothers, largely attributable to premenopausal breast cancer, where a 3-fold excess above expectation was observed [9, 10].

This group went on to trace the families of an extended series of children with sarcomas included in their population-based registry and they reported on the cancer experience among all first-degree relatives of the eligible children. A statistically significant excess of cancers was found among all first degree relatives (relative risk, RR = 1.6). This was largely accounted for by cancers in mothers and siblings with no excess observed among the fathers. The excess of cancer was due mainly to carcinoma of the breast and cancers in children. The risk was highest for cancers diagnosed at younger ages. In order to try to identify families at particularly high risk of cancer, these workers carried out multivariate analysis of clinical characteristics in the index patient. This analysis identified young age at diagnosis, histological subtype of embryonal rhabdomyosarcoma, and male sex as independent indicators of elevated risk of cancer in first degree relatives [11].

Results of the study of families of the hospital-based series of survivors of childhood soft tissue sarcoma were largely consistent with the above study. Among the first degree relatives of children included in this series, 34 cancers occurred, compared with nearly 21 expected, with the excess predominently accounted for by breast cancers and sarcomas diagnosed at young ages. Relatives at highest risk in this series were those of children with soft tissue sarcoma diagnosed at young ages, histological type of embryonal rhabdomyosarcoma and of children with multiple primary cancers [12]. Therefore, although the index patients were selected according to different criteria, and were from different countries, the results of the above two studies were remarkably similar.

Williams and Strong, and Lustbader and associates conducted segregation analyses in the hospital-based series, which demonstrated that the cancer distribution in the families was compatible with a rare autosomal-dominant gene (gene frequency = 0.00002). The penetrance was estimated to be almost 50% by 30 years and 90% by 60 years. The relative risk of affection in children who carried the gene was estimated to be 100 times the background rate. The age-specific penetrance was somewhat higher in females due to the occurrence of breast cancer, but maternal and paternal lineages equally contributed to the evidence favouring a dominant gene [13, 14].

Li and colleagues collected detailed information on 24 kindreds in order to study the characteristic components of the syndrome [15]. To be eligible for the study, each kindred had to conform to the following criteria: bone or soft tissue sarcoma diagnosed under 45 years of age in an individual who was then designated the proband; one first-degree relative of the proband with cancer under 45 years of age and one first- or second-degree relative in the same lineage with cancer under 45, or sarcoma diagnosed at any age. Subsequently these criteria have found wide acceptance as a clinical definition of the syndrome, and families conforming to these criteria are referred to hereafter as having "classic" LFS. Two families with classic LFS are illustrated in Figures 1 and 2, based on probands with childhood and adult onset sarcoma, respectively.

Among the 24 kindreds, 151 blood relatives had developed cancer. Of these, 119 (79%) were affected below the age of 45, compared with 10% of all cancers in this age range in the general population. With regard to the distribution of cancer types, in addition to bone and soft tissue sarcoma and breast cancer, it

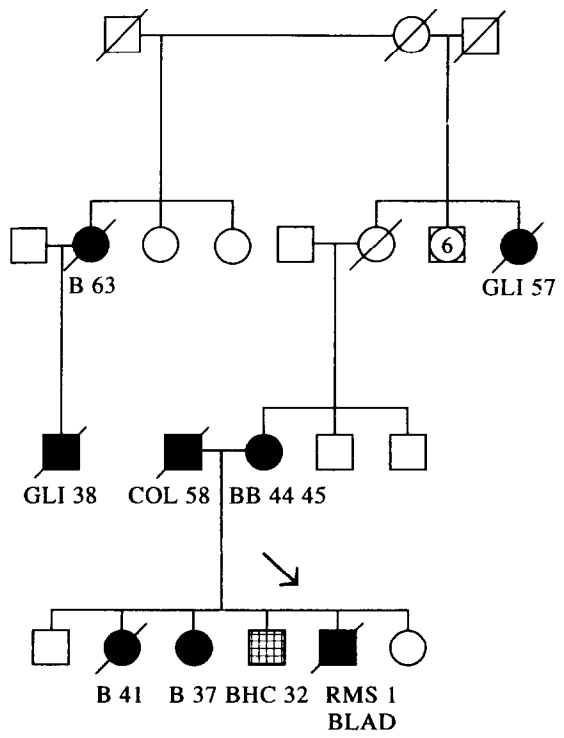

Figure 1. A family classic LFS in which the proband was diagnosed with embryonal rhabdomyosarcoma of the bladder in early childhood. B, carcinoma breast; BB, bilateral carcinoma breast; COL, carcinoma colon; GLI, glioma; RMS BLAD, rhabdomyosarcoma bladder; BHC, benign histiocytoma; OS, osteosarcoma; FEM, femur; MAND, mandible; LMS, leiomyosarcoma; CS, chondrosarcoma; VERT, vertebrae; RCS, reticulum cell sarcoma; UK, carcinoma, unknown primary site; ●, female with cancer; ○, female without cancer; ■, male with cancer; □, male without cancer; /, deceased; →, proband.

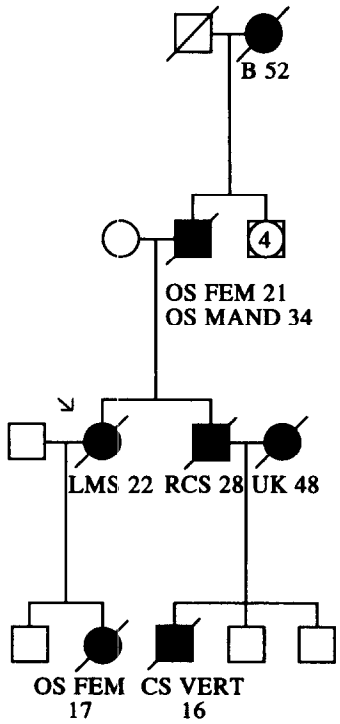

Figure 2. A family with classic LFS in which the proband was diagnosed with leiomyosarcoma of the buttock as a young adult. See Figure 1 for abbreviations.

was found that brain tumours, adrenocortical carcinoma and leukaemia also were in excess compared with U.S. population-based cancer incidence data. Multiple primary cancers had occurred in 15 individuals, and the types of cancers which emerged as the principal components of the syndrome, based on first primary cancers, were also the principal types among second and subsequent primaries [15]. In addition to confirming bone and soft tissue sarcomas, breast cancer, brain tumours, leukaemia and adrenocortical carcinoma as the main component cancers associated with LFS, the population-based and hospital-based studies referred to above also suggested that melanoma, germ cell tumours and Wilms' tumours may represent other syndrome-associated cancers [11, 14, 16–18].

These systematic studies provided substantial evidence for inherited susceptibility to a diverse but specific spectrum of cancers within certain families. The syndrome had thus been well characterised statistically and with regard to cancer phenotype. Identification of the gene responsible for cancer susceptibility in LFS families was more difficult. Whereas associated constitutional chromosomal abnormalities had indicated the probable locations of the retinoblastoma and Wilms' tumour genes, no such characteristic aberrations had been found in any of the families with the syndrome. Furthermore, genetic linkage analysis was not possible, because of the small number of suitable families available. Genetic marker typing of sufficient numbers of affected family members was a problem because of the lethal nature of the component LFS cancers. Because some of these component cancers are common in the general population, the possibilty of phenocopies also arose.

For these reasons, Malkin and colleagues [19] chose to analyse candidate genes. On the assumption that the gene responsible for LFS was likely to be a tumour suppressor gene, they selected the *TP53* gene, located on chromosome 17p13, as a possible candidate. Deletions and/or mutations in this gene had been found in DNA extracted from tissue derived from sporadic cancers associated with LFS, including osteosarcomas, soft tissue sarcomas, brain tumours, carcinoma of the breast, and leukaemias. Furthermore, transgenic mice carrying mutated *TP53* had been shown to develop a high incidence of malignancies including bone and soft tissue sarcoma [20].

Normal tissue samples from affected members of five LFS families and unaffected members from one of the families were, therefore, analysed for the presence of *TP53* germline mutations. Such mutations were detected in all affected individuals examined. In the family where samples from unaffected members were also analysed, the unaffected grandfather of the proband, who was aged 57 years at the time of the study, and the first cousin of the proband, who was aged 5 years, were also found to carry a germ line *TP53* mutation. The mother of the latter individual had developed bilateral breast cancer at the age of 28 years. These two individuals were regarded as being at high risk of developing LFS-associated cancers. The germ line mutations detected in these families affected codons 245, 248 (two families), 252 and 258 of the *TP53* gene. A sixth LFS family with a germ line mutation in codon 245 was reported soon after this initial publication [21].

The *TP53* gene is present in all vertebrate species so far examined, and in particular there are five domains within the coding region of the gene which have been highly conserved during the course of evolution. These are referred to as highly conserved domains (HCD) 1-V spanning codons 13–19, 117–142, 171–181, 236–258 and 270–288, respectively. *TP53* mutations in sporadic tumours have been found throughout the coding sequence, but the majority cluster in HCDs II-V, with codons 175, 248 and 273 being most commonly affected and codons 245 and 282 rather less frequently. These codons can be regarded as mutational hot spots [22].

It can be seen that the six germ line mutations originally reported had all occurred in a stretch of 14 codons within HCD IV, encompassing part of exon 7. Subsequently, there was much speculation about the possible significance of this positional clustering, with the suggestion that the types of *TP53* mutations which could occur constitutionally may be restricted, and other mutations may be lethal [23].

A period of intense research activity then followed, with several groups throughout the world analysing material from families and patients thought to be candidate carriers of *TP53* germ line mutations. These included families with classic LFS, families with patterns of cancer suggestive of LFS (LFS-like families), site-specific early-onset breast cancer families and patients with LFS-associated cancers, regardless of family history, including bone and soft tissue tissue sarcoma, premenopausal breast cancer and patients with multiple primary cancers. A number of single family case reports appeared, in which germ line mutations in *TP53* had been detected in members of LFS or LFS-like families [24–27]. Sameshima and associates [28] identified two families with germ line *TP53* mutations through young children with adrenocortical carcinoma. Both families showed an LFS pattern of cancers. These six families included examples of germ line mutations in exons 5 and 8, as well as in exon 7. Larger series of families with features of LFS were analysed by Brugières and colleagues and Birch and associates [29, 30].

The series of Brugières and colleagues consisted of ten families of children with solid tumours, eight with sarcomas and two with neural tumours, where at least one first- or second-degree

relative of the probands had been diagnosed with cancer before 45 years of age. The series included five examples of classic LFS, three families in which the proband had an affected first-degree relative (incomplete LFS) and one family with three affected relatives, but not fulfilling the definition for classic LFS (LFS-like). In the final family, a second-degree relative of the proband had developed childhood cancer. Germ line mutations in exon 8 of *TP53* were found in two of the families with classic LFS, and a mutation in exon 7 was found in one family with incomplete LFS. In this study, however, only exons 5–8, which include HCD II, III, IV and V were analysed. It is possible, therefore, that germ line *TP53* mutations in other parts of the coding region of the gene may have been present in other families in the series.

The series of Birch and associates included 12 families with classic LFS and nine LFS-like families which had been systematically ascertained through patient registers of children with cancer and adults with bone or soft tissue sarcoma. In this study, the entire coding sequence of the *TP53* gene was analysed for the presence of germ line mutations. Such mutations were detected in seven of the families, six among those with classic LFS and one among the LFS-like families. The mutations occurred in exons 4, 5, 6 and 7, and included mutations outside of the HCDs, and complex deletion-insertion mutation as well as point mutations. Germ line *TP53* mutations in this series appeared to be associated with families which included young children with rhabdomyosarcoma and/or adrenocortical carcinoma.

Three groups of workers have analysed series of breast cancer patients and breast cancer families for the presence of germ line *TP53* mutations. Prosser and colleagues [31] found 1 patient with a constitutional mutation in codon 267 among an unselected series of 136 breast cancer patients. The proband had developed breast cancer at age 49 years and investigation of her family history showed that her mother had developed lung cancer and cervix cancer at the ages of 53 and 62, respectively. Her maternal grandmother and maternal great aunt had both had breast cancer at the ages of 42 and 67 years, respectively. The daughter of the latter patient had developed ovarian cancer at 62 years of age. This family, therefore, did not display a pattern of cancers consistent with LFS, and the ages at onset of cancer were markedly later than in previously reported families with germ line *TP53* mutations. No samples were available for analysis from affected members of the family other than the proband, but two unaffected relatives were found to be carriers of the mutation, and at the time of analysis were alive and well at the ages of 37 and 74 years, respectively. The mutation had been observed only once before in a sporadic cancer, and occurs outside of the conserved domains [22]. It would seem, therefore, that this mutation is either of low penetrance, or alternatively may represent a polymorphic variation of no biological significance.

In a study by Sidransky and colleagues, samples from 126 consecutive patients with breast cancer diagnosed up to the age of 40 years were analysed for the presence of constitutional *TP53* mutations in exons 5–8. One of these patients was found to have such a mutation in codon 181. Her mother was observed to have had bilateral premenopausal breast cancer and her grandmother, who had a history of carcinoma of the colon, had developed breast cancer at the age of 72 years. The proband, whose breast cancer was diagnosed at the age of 33 years, developed a second cancer, a melanotic spindle-cell carcinoma of the mediastinum, at the age of 35 years [32]. Børreson and associates [33] reported results of an analysis of constitutional samples from an unselected series of 167 breast cancer patients, 40 patients who had developed breast cancer under 35 years of age, and 30 breast cancer patients who had a family history of breast cancer. In this study, exons 5–8 of the *TP53* gene were analysed. 2 patients with germ line mutations in codon 181 in a patient from the unselected series, and codon 245 in a patient with early onset breast cancer, respectively, were identified. Both of these patients had family histories of cancers, which, although not fulfilling the definition of classic LFS, were consistent with the syndrome.

Toguchida and associates [34] analysed exons 2–11 of the *TP53* gene in constitutional samples from a series of 196 patients with bone or soft tissue sarcoma. 181 of these patients were completely unselected (sporadic group), but 15 patients were selected because of an unusual family history of cancer, or the presence of multiple primary tumours. 3 patients with osteosarcoma in the sporadic group were found to have germ line *TP53* mutations, but although these patients were from the unselected series, 2 had developed multiple primary tumours, and the young daughter of one of these had also developed a soft tissue sarcoma. The third patient had a sibling with osteosarcoma. In the selected series of 15 patients, germ line mutations were detected in five. These eight germ line *TP53* mutations involved exons 4, 5, 6, 7 and 8, and included non-sense as well as missense mutations. The personal and family histories ofthese 8 patients were all consistent with LFS.

The coding sequence of *TP53* was analysed in samples from 25 children with acute lymphoblastic leukaemia by Felix and associates [35]. One of these patients had a family history consistent with classic LFS, and in this patient, a germ line mutation in exon 8 of *TP53* was detected.

Multiple primary tumours are a feature of LFS, and Malkin and colleagues investigated 59 children and young adults, who had developed second malignant neoplasms, for the presence of germ line *TP53* mutations. The analysis included the four HCDs encompassing all the mutational hot spot regions of the *TP53* gene. 4 of these patients were found to carry germ line mutations in exons 7, 8 (2 cases) and 9. The family histories in 2 of these patients were consistent with LFS, and breast cancer and colon cancer at the ages of 57 and 63 years had been diagnosed in close relatives of the remaining 2 cases, respectively [36]. A mutation in exon 8 of *TP53* was found in 1 of 4 patients with multifocal osteosarcoma analysed by Iavarone and associates [37], although there was no significant family history of cancer in this patient.

From these studies, it appears that germ line mutations in the coding region of the *TP53* gene are a frequent but not universal finding among families with classic LFS, or with patterns of cancer consistent with the syndrome. However, among patients with LFS-component cancers, the incidence of germ line *TP53* mutations is very low indeed, in the absence of family histories of cancer, or the presence of multiple primaries or multifocal disease.

The distribution of these 40 published germ line coding mutations in the *TP53* gene is shown in Figure 3. Sixteen of these have occurred in exon 7, including eight affecting codons 248 and four affecting codon 245. Twelve mutations occurred in exon 8, including four at codon 273 and four at codon 282. Six mutations occurred in exon 5 and two in exon 6. Only four mutations have been identified outside exons 5–8. However, these may be underrepresented, since only the conserved regions of *TP53* have been analysed by most investigators. It can be seen from Figure 3 that the great majority of germ line mutations so

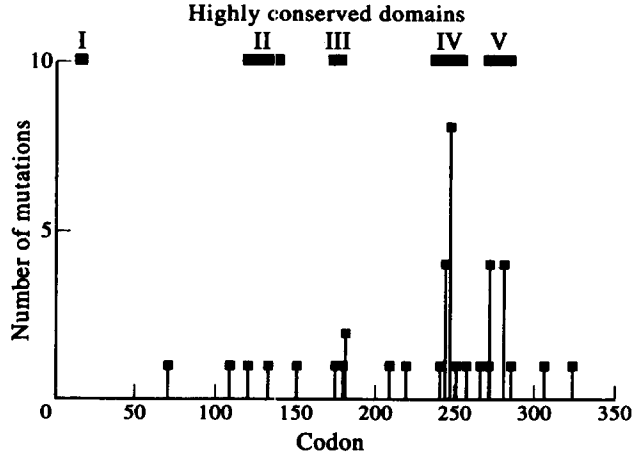

Figure 3. Distribution of germ line mutations in the coding region of the *TP53* gene, indicating the relative positions of the highly conserved domains (HCD) within the gene. The majority of mutations occur within HCD IV and V.

far identified occur within HCDs III, IV and V. But in contrast to the marked positional clustering within part of exon 7, observed in the six *TP53* germ line mutations originally reported, the distribution now more closely resembles that found in mutations detected in sporadic tumours. Nevertheless, conserved region IV within exon 7 has clearly emerged as a hot spot region for germ line mutation.

Of these 40 published constitutional *TP53* coding mutations, 29 are transitions, of which 21 occurred at CpG dinucleotides. Only six transversions were seen, with five examples of deletion and/or insertion mutations. It is of interest that in sporadic breast cancers, transversions and transitions are found with equal frequency. Although carcinoma of the breast is the most common cancer occurring in adult members of LFS families, the distribution of types of *TP53* mutations occurring in the germ line more closely resembles that found among sporadic colon cancers [38]. Carcinoma of the colon has been observed in families with constitutional *TP53* mutations, but is not a frequent occurrence in these families. The hypermutability in CpG dinucleotides arising from the spontaneous deamination of 5-methylcytosine is well documented [22]. Since more than half of the published examples of germ line coding mutations are of this type, this suggests that the majority may arise as a result of spontaneous events. Exogenous agents may thus play only a minor role in the generation of *TP53* germ line mutations.

The conserved exons of the *TP53* gene only were analysed in the majority of the studies referred to above. Therefore, the possibility of mutations occurring in other regions of the gene exists, and three examples of intronic mutations have been published. The first of these was reported by Warneford and associates [39], who detected a point mutation in the splice donor site of intron 4, in constitutional samples from two affected members of a family with an extensive history of cancer consistent with LFS. In this family, a mother and son representing joint probands had both developed adrenocortical carcinoma, with the mother developing breast cancer at 35 years of age as a second primary. The mutation led to an aberrant larger transcript which was detected in both tumour and constitutional material in addition to the normal transcript.

The second example was found in a patient with osteosarcoma occurring during childhood and an unusual steroid-secreting abdominal tumour with onset during young adulthood. The mutation detected in intron 5 consisted of the deletion of an 11 base-pair sequence which involved a region of splicing recognition. This mutation resulted in the deletion of exon 6, creating a frameshift and premature stop codon in exon 7 [40]. Recently, another example of a splice-site mutation of the *TP53* gene in the germ line in a cancer family has been published [41]. This is of particular interest, since the family was characterised by hereditary breast and ovarian carcinoma. This familial pattern of cancers is frequently linked to a gene on chromosome 17q designated *BRCA1* [42]. However, the presence of choroid plexus carcinoma in a young child who was the proband, and the very early onset of the breast and ovarian cancers in this family, is also consistent with LFS. The germ line mutation involved a single-base substitution in intron 5, affecting the splice acceptor site, resulting in deletion of exon 6 and creation of a frameshift leading to a premature stop in exon 7. The findings in these families suggest that intronic mutations may account for the cancer susceptibility in at least some of those families with classic LFS in whom the presence of germ line *TP53* coding mutations could not be detected.

The above review demonstrates that, although the pattern of cancers, associated with germ line *TP53* mutations in families and individual patients with multiple primary cancers is by and large consistent with LFS, the cancer phenotype is broader than that associated with "classic" LFS. While breast cancers and sarcomas are common findings in patients and families with germ line *TP53* mutations, a very wide spectrum of cancers, which includes most of the common carcinomas of adulthood as well as rare paediatric malignancies, has been observed. Penetrance also appears to be very variable, with cancers occurring in infancy and multiple primary cancers occurring in some individuals, whilst, in contrast, other individuals carrying such mutations, have survived into old age without developing malignant disease, even within classic LFS families. The issue of what factors might influence penetrance and phenotypic expression therefore arises.

Whether or not specific germ line *TP53* mutations are more penetrant and associated with different cancer phenotypes, compared with others, has not yet been resolved. At present, studies of possible correlations between type of mutation and cancer phenotype are not possible, as too few families have been fully characterised, even on an international basis. *TP53* can be classified as a tumour suppressor gene, but certain mutant protein products are associated with a gain of function or transdominant effect on the wild-type *TP53* product, and phenotype is influenced at the cellular level [43, 44]. If such gain of function mutations occur in the germ line, these may confer a more highly penetrant cancer phenotype with respect to lifetime risk of cancer, age at onset, and probability of developing multiple primary cancers, than mutations resulting in a simple loss of function of the wild-type protein.

The influence of external factors on the development of malignancy in carriers of germ line *TP53* mutations is also unknown, but it would be expected that exposure to occupational or environmental carcinogens, social habits, and other lifestyle characteristics may contribute to the determination of the specific type of cancer and the age at which this develops. The apparent wide variations in morphological types of cancer and ages at diagnosis observed in carriers of identical mutations, even within the same family, may be explained by differences in such lifetime experiences in combination with the genetic background of a particular individual. These issues can only properly be addressed by planned prospective follow-up studies

of large numbers of families with germ line *TP53* mutations. Results of such studies, in combination with a deeper understanding of the normal function of *TP53* and its molecular partners, will have profound implications for the clinical management of such families, including treatment in those individuals developing cancers and intervention measures, including chemoprevention, in unaffected individuals and survivors of initial cancers.

Current studies indicate that approximately 50% of families with patterns of cancer consistent with classic LFS do not have constitutional *TP53* coding mutations. A proportion of these families may have intronic or promoter region mutations. In other families, a constitutional mutation in another gene which interacts with and compromises the normal function of wild-type p53 protein may occur. This type of situation is suggested by two families in whom the pattern of cancers is consistent with LFS, but where no germ line mutation in *TP53* was detected. In tissue samples from affected members of both these families, immunohistochemical analysis, using the polyclonal anti-p53 antibody CM1, detected increased expression of apparently wild-type p53 protein in normal tissue as well as tumour tissue [45, 46]. Direct involvement of *TP53* has been excluded in one of these families by linkage analysis [46]. In this latter family, other candidate genes including *BRCA1* and *MDM2* were also considered, but were similarly excluded by linkage analysis. The basis of cancer susceptibility in LFS families, negative for the presence of germ line *TP53* mutations, is a current area of particular research interest. It is likely that other genes conferring a LFS pattern of cancers in families will be important in the histogenesis of many common cancers including breast cancer.

In those families with germ line *TP53* mutations, it is technically feasible to test asymptomatic members for the presence of these mutations. However, such predictive testing presents a number of ethical, technical and clinical difficulties which must be addressed. The prevalance of germ line *TP53* mutations among the general population of cancer patients appears to be extremely low, and even among patients with component LFS cancers, the frequency of constitutional mutations in *TP53* appears to be around 1% [31, 33, 34]. Screening the general population of patients with these cancers for the presence of *TP53* germ line mutations would, therefore, be inappropriate, and such testing should be confined to the close relatives of individuals shown to carry a constitution *TP53* mutation. Such individuals would probably be members of LFS families or patients who have developed multiple primary cancers associated with LFS.

Estimation of cancer risks in carriers of these mutations for the purposes of counselling presents difficulties, since the morphology-, site-, age- and sex-specific incidence of malignancy in mutation carriers are unknown. Since paediatric cancers are a frequent occurrence in families with classic LFS, the problem of whether it is ethically acceptable to test healthy children for the presence of constitutional *TP53* mutations arises. Such testing could be justified only if it could be shown that screening for early detection of malignant disease in such children would confer a benefit in terms of survival or reduction in morbidity. There is no such evidence that this is the situation at present.

Because of the wide spectrum of adult-onset cancers which have occurred in carriers of *TP53* germ line mutation, it is similarly problematical to envisage a screening programme which could be effective in detecting these cancers. This is the situation even with respect to early detection of breast cancer, the most common adult onset cancer in LFS families. Mammography is of unproven benefit in premenopausal women, and more than 30% of breast cancers in carriers of germ line *TP53* mutations were diagnosed before 30 years of age and 97% before age 50 years [30]. A further difficulty of predictive testing for these mutations is the current poor understanding of the psychological impact on individuals of giving them the knowledge that they are at increased risk of developing cancer, and that the risk could be or may have been transmitted to their children. The psychosocial aspects of predictive genetic testing for cancer susceptibility should therefore also be addressed in an investigative manner in any predictive testing programme [47–49].

In conclusion, the delineation of the Li–Fraumeni syndrome and the finding of constitutional mutations in the *TP53* gene in families illustrate how clinical observations in patients with rare childhood cancers, and painstaking epidemiological research have come together with biochemical and molecular biological research to produce results of wide-reaching importance both in clinical and scientific spheres of cancer research.

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