

An extended Li-Fraumeni kindred with gastric carcinoma and a codon 175 mutation in TP53

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Abstract

We present an extended family with Li-Fraumeni syndrome characterised by gastric and breast carcinoma, glioma, sarcoma, and leukaemia. This family showed strong evidence of linkage to TP53, and three of four tumours analysed showed loss of the wild type allele. A codon 175 missense mutation was identified in exon 5 in all available affected subjects. Counselling, screening, and issues surrounding presymptomatic testing are discussed.

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It has been known for some time that common malignancies can cluster together in a fashion indicating that hereditary factors are involved. Furthermore the existence of site specific breast,¹ colon,² and ovarian³ cancer families showing autosomal dominant inheritance have long been described. Cancers may also segregate together, for example, breast and ovary⁴ and endometrium and colon.⁵ The latter group, hereditary non-polyposis colorectal cancer,⁶ also includes cancer of the ovary, pancreas, and breast. The combination of childhood soft tissue sarcomas and early onset of breast cancer in mothers and other female relatives, together with adrenocortical tumours, brain tumours, osteosarcoma, and leukaemia occurring to excess, is also a recognised syndrome.⁷ This is known as the Li-Fraumeni syndrome (LFS) and was first described in 1969.⁸ Germline mutations involving the TP53 gene have been shown to account for the majority, but not all, of classical LFS families.⁹⁻¹² We describe an LFS family unusual for the presence of two gastric carcinomas and in which the structure of the family was sufficient to establish linkage

to TP53. Subsequent DNA sequence analysis showed a missense mutation in codon 175 in exon 5 of TP53 in the family.

Patients and methods

The family (figure) was ascertained through the mother of IV·5 who had asked for counselling on the child's cancer risks. At about the same time, III·5 also presented, worried about the recent death of his brother (III·2). Initially it was only possible to ascertain affected cases up to II·2; however, after two years of extensive research, the full extent of the tumours in this branch of the family was established (III·8, III·13, IV·6). Diagnoses of the tumours were confirmed through death certificates, hospital records, and pathology reports and these were further checked by using the relevant cancer registry. Confirmation of histology was possible in all cases except I·1 and II·3. The diagnosis and age at presentation and survival of each case is presented where known (figure, table). There were no dermatological or other phenotypic markers on examining affected subjects.

Molecular studies

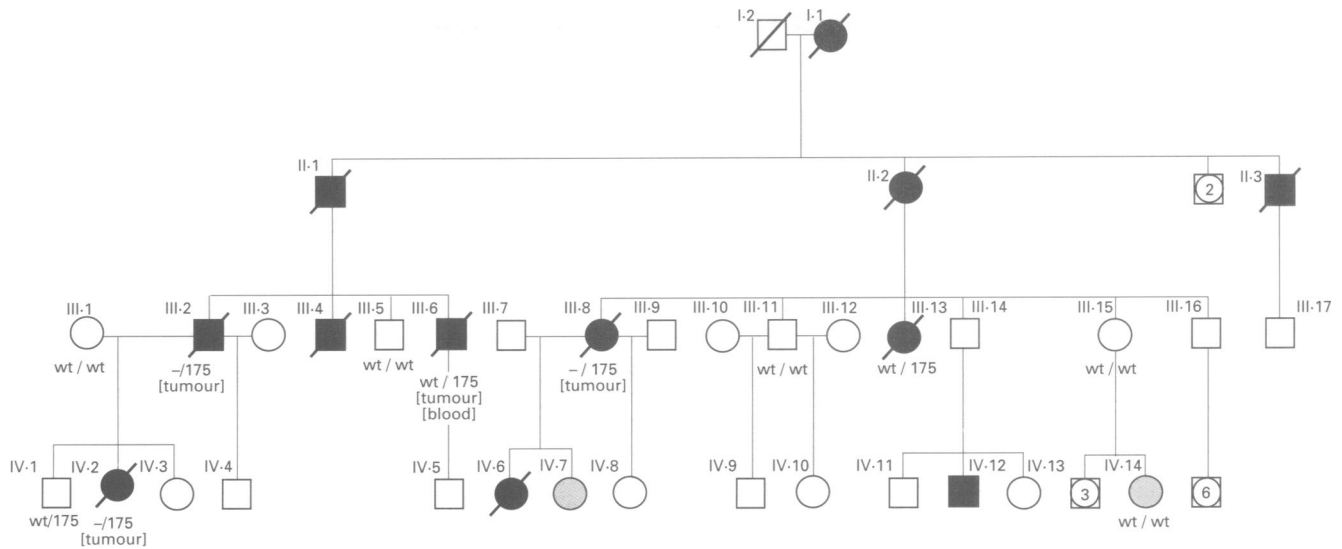
Initially no living affected family member was identified, therefore tumour (paraffin block) material was obtained from three subjects who had died (III·2, III·6, and IV·2), enabling analysis of DNA from affected subjects. More recently, a blood sample from a living affected subject (III·3) has become available, plus tumour material from III·8 and blood from unaffected subjects (III·1, III·5, III·11, III·15, and IV·1; all given for research purposes only). Normal cervix and CINII/III tumour material were obtained from IV·14. DNA samples were therefore available from 11 family members, five affected and four at 50% risk.

Initial studies of the TP53 gene involved mutation detection screening of DNA from the tumour of IV·2 using the HOT technique, which failed to detect a mutation.¹¹ A polymorphic dinucleotide microsatellite repeat mapping within the TP53 gene¹³ was used to analyse segregation of TP53 alleles through the family, and samples from III·2, III·5, III·6, III·11, III·13, III·15, IV·1, and IV·2 were studied. A shared TP53 allele was identified in III·2, III·6, III·13, IV·1, and IV·2, all of whom were affected with the exception of IV·1. The linkage data indicated that a mutation within or close to the TP53 gene could be the causative defect, and that IV·1 was an unaffected carrier. Lod scores were calculated using penetrance

Tumour diagnoses and survival in family 117 with Li-Fraumeni syndrome

Subject	Tumour diagnosis	Age (y)	Survival from diagnosis (mth)
I·1	Brain tumour (not confirmed)	42	NK
II·1	Gastric adenocarcinoma	35	0
II·2	Breast carcinoma (left)	37	1
II·3	Laryngeal carcinoma (not confirmed)	21	NK
III·2	High grade glioma	34	<1
III·4	Chondrosarcoma (pelvis)	16	12
III·6	Dermatofibroma	31	
	Clear cell gastric adenocarcinoma	35	1
III·8	Astroblastoma brain	29	41
	Breast carcinoma (right)	32	8
	Lipoma	32	
III·13	Astrocytoma grade II/III brain	31	33
IV·2	Adrenal cortical adenoma (virilising)	2	<1
IV·6	Chondrosarcoma left iliac bone	11	9
IV·12	Acute lymphoblastic leukaemia	13	12*

* Still living but in relapse.
Mean survival from diagnosis, where known, 11·2 months.



Pedigree of family 117.

probabilities as described¹⁴ and were estimated at 1.24 ($\theta=0$) without incorporating the loss of heterozygosity (LOH) data (see below) and 2.17 ($\theta=0$, $\rho=0$) including the LOH data.^{15,16}

When further DNA samples became available from affected and unaffected subjects, we sequenced the entire coding region in two subjects (III-8, III-13) using Sequenase dye-terminator chemistry and an ABI model 373 sequencer, and previously published primer sequences to analyse the entire gene exon by exon.¹¹ An identical missense mutation was identified in exon 5 in DNA from blood from III-13 and tumour from III-8. This mutation was within codon 175 (CGC→CAC, Arg→His), and altered a recognition site for the endonuclease *Hha*I. Amplification of exon 5 followed by digestion with *Hha*I allowed resolution of wild type from mutant alleles and permitted us to analyse rapidly all the available DNA samples from the family. Direct sequencing of exon 5 was used to confirm the presence or absence of a mutation. Of the samples available, six showed a mutation (III-2, III-6, III-8, III-13, IV-1, and IV-2) confirming the linkage data. Five people did not have the mutation (III-1, III-5, III-11, III-15, and IV-14). III-5, III-11, and III-15 had previously been assessed at 50% risk, although linkage to TP53 had indicated that they were unaffected carriers.

DNA from four tumours were available; three showed loss of the wild type allele (III-2, III-8, and IV-2).

Counselling issues

Involvement of this family in research to identify the causative gene has stimulated one of two responses. Of the people initially at 50% risk, III-5, III-11, III-15, IV-1, IV-3, IV-5, and IV-8 have all been referred for formal genetic counselling. Standard genetic counselling was offered, including estimation of risk. However, IV-7 and III-14 have refused to take part even in the research aspects of the study. Even after the presentation of his son (IV-12) with an

unusual leukaemia, III-4 continued to prefer not to become involved in the research or to have genetic counselling. Thus samples from IV-12 have not been available for study.

Screening

Subjects at risk have been offered open access to the genetics service. There is a very low threshold for extensive investigation of any abnormal symptom or sign. In addition, children are offered an annual ultrasound scan of the abdomen and pelvis, and a full blood count and film, while adults are offered a general physical examination. Breast screening in women involves an annual breast examination and ultrasound scan, but with a baseline mammogram at 25 years. One person (III-5) has opted for three yearly upper GI tract endoscopy.

Discussion

The family studied has early onset tumours in four generations, colon and endometrial cancer are absent, and the malignancies have been particularly severe. Two people developed a childhood sarcoma, which is classical of LFS, as is the presence of brain tumours in four subjects. An increase in the risk of breast cancer in mothers of children with osteosarcoma and chondrosarcoma has been reported,¹⁷ and is shown in this family. The presence of an adrenal cortical adenoma is also characteristic of LFS families, although most reports specify adrenal cortical carcinoma. The development of two gastric and one laryngeal cancer at young ages in this family is unusual for LFS. A report of tumours in 43 LFS families contained only four gastric carcinomas out of a total of 231 tumours.⁹ Previous reports in which gastric tumours feature in the pedigree¹⁸ can be confusing, as the cancers occur in the branch of the family assumed not to be involved. However, it is clearly a component of some germline TP53 mutation families. A codon 282 mutation in exon 8 has been described in a family with four cases of gastric carcinoma,¹⁹ and in the present

report we have shown the presence of the codon 175 mutation in the family, including in DNA from a patient with clear cell gastric adenocarcinoma (III-6). Three of the four tumours reported here have shown loss of the wild type allele, suggesting that the mutant TP53 is functioning as a tumour suppressor gene in this family.

The family reported here (family 117) has been part of a previous study in which it was described as one of the 50% of 12 classical LFS families in which a mutation in TP53 was not found.¹¹ This may have been a consequence of the non-availability of DNA from a living affected subject at the time of the original study, and the use of tumour DNA for the mutation analysis. The adrenal cortical adenoma used in the original study appears to have lost the wild type allele, and it may therefore not have been possible to identify the missense mutation using the HOT technique, which detects mismatches between mutant and wild type sequences. Alternatively, the HOT technique may have been unable to detect this particular type of mutation. With the subsequent availability of other DNA samples from family members and the apparent linkage to TP53, we were prompted to sequence the entire coding region, leading to the identification of the causative mutation. We have previously noted that among LFS families the presence of an adrenal cortical carcinoma in a young child appears to confer a high chance of carrying a TP53 mutation in the germline.¹¹ The finding of the codon 175 mutation in this family, which includes an infant with an adrenal cortical adenoma, adds weight to this observation.

Family 117 is one of the largest LFS families reported with 12 affected subjects and a further one obligate carrier (III-14). It is rare to have a family sufficiently large and with sufficient affected subjects on whom DNA is available to undertake linkage. While only 50% of classical LFS families have an identifiable reading frame mutation, it is thought that up to 85% involve TP53.²⁰ This may be because of promoter sequence mutations or other mechanisms that may only be detected by allele specific expression studies. There are clearly LFS families in which coding mutations within TP53 have been excluded^{21,22} and therefore a sizeable lod score would have to be generated to offer predictive testing based on linkage to TP53 alone. Using Easton's model²³ for predictive testing for BRCA1 linkage in breast-ovary families, it would be necessary to generate a lod score in excess of 1.0 if mutation studies had not been carried out, and of 1.5 if they had (and no mutation found), to obtain 95% confidence with informative flanking markers. This would take into account that up to 40% of LFS families without a detectable coding TP53 coding sequence mutation were nevertheless associated with an inherited TP53 gene abnormality. If the LOH data were not incorporated into the linkage calculation, the lod score was 1.24 ($\theta=0$), although inclusion of LOH data raised this to 2.17 ($\theta=0$, $\rho=0$). Even in a large family such as this it is difficult to obtain material on enough relatives to achieve

sufficient power to detect linkage. TP53 is a tumour suppressor gene, and so loss of the wild type allele in a tumour is further evidence for TP53 involvement. Thus, allele loss in an affected carrier can be treated as an additional meiosis. Inclusion of such LOH data is particularly suitable in Li-Fraumeni families where many affected cases have multiple primary tumours, each of which can be treated as an independent event (meiosis). However, offering a linkage diagnosis could be problematical and is still based largely on assumptions. In the present family, we were uncomfortable offering linkage diagnosis even though there was considerable pressure for us to do so, but with the identification of the causative mutation, we have a robust diagnostic test.

Although five members of this family have already expressed a very strong interest in knowing their TP53 status, predictive testing must be offered with caution. The identification of carrier status would imply a near 90% risk of malignancy by 50 years of age,¹¹ which in this family has resulted in death within a year in the majority of affected people. There is an understandably deeply entrenched fear of cancer in this family and in the absence of proven benefit from intervention, even genetic counselling should not be forced upon family members. III-14, who would be identified as a gene carrier in counselling, may cope better psychologically by using denial, even if he feels he carries the mutation. Experience with predictive testing in Huntington's families according to a standard protocol has shown a low uptake for testing.^{24,25} Use of a standard protocol for predictive testing in potential carriers of TP53 mutations has been recommended.²⁶⁻²⁸ It is likely that if there is careful counselling a similarly low uptake for testing will occur in Li-Fraumeni families. At present, while there is no evidence that screening confers a benefit to children with LFS, predictive testing in childhood as in Huntington's disease is probably inadvisable.^{24,26-28} There are, however, a number of subjects who would at counselling be at 50% risk, but who would not carry the mutation. Benefits for these people may be considerable.

An accepted protocol for disease screening in LFS families has yet to be devised. The evidence from TP53 deficient mice for susceptibility to the effects of radiation²⁹ means that mammography (or other radiological screening such as CT) should not be used as a regular tool. However, ultrasound is even less effective than mammography in identifying early malignant lesions in the breasts of young women. The risk of breast cancer in LFS would justify some form of screening from 20 years of age (30% of breast cancers occur at <30 years in TP53 mutation carriers¹¹), and magnetic resonance imaging may well be the way forward. However, the range and diversity of site of the tumours in LFS mean that even a total body MRI may not be sufficient to have an effect on mortality. The short survival as a consequence of the aggressive nature of the tumours in this and other LFS families may mean that the lag time to symptoms is very

small. Nonetheless, the pressure from family members to have at least some form of screening is often great.

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