

Low rate of *TP53* germline mutations in breast cancer/sarcoma families not fulfilling classical criteria for Li-Fraumeni syndrome

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Breast cancer and sarcoma are key components of Li-Fraumeni syndrome (LFS).^{1–6} Sarcoma, particularly childhood osteosarcoma or rhabdomyosarcoma in addition to childhood adrenocortical carcinoma (ACC), is the strongest predictor of the presence of a *TP53* mutation.^{7,8} However, while up to 80% of unselected series of ACC have *TP53* germline mutations,⁸ only 3–10% of unselected sarcomas have been found to have such mutations.^{9–11} At least half of these would have been predicted on the basis of family history and many of the rest could have arisen de novo.¹² While breast cancer is common in LFS and the penetrance of *TP53* germline mutations in women for breast cancer may be as high as 56% by the age of 45 years (80% of female cancer incidence aged 16–45 years),^{13,14} it is also common in the general population with nearly 2% of women now developing breast cancer by the age of 50 in the general population in the western world.^{15,16} In contrast to sarcoma and ACC, there are other more common inherited syndromes to account for familial aggregation of breast cancer (*BRCA1/2*). As a major referral centre for research testing for *TP53*, we have become aware that the possibility of *TP53* mutations is often raised fairly strongly in the context of even a single case of sarcoma in addition to breast cancer. In order to assess the likelihood of *TP53* germline mutations in this population, we have assessed the outcome of such testing in families containing a single (but no more) sarcoma and at least one breast cancer where the family as a whole does not fulfil LFS criteria.

MATERIAL AND METHODS

Over the last 20 years our group has ascertained families with a history of early onset tumours in addition to sarcoma.^{4–6,17} In the last 10 years we have also received samples from families ascertained at other genetics and oncology centres in the UK. We have retrospectively analysed the outcome of *TP53* germline mutation testing in families with a single proven sarcoma where that person or a first degree relative developed early onset (<60 years) breast cancer, but the family as a whole did not fulfil criteria for classical LFS (table 1). Twenty-one such families were identified (table 2) in addition to 30 families which we had tested that fulfilled classical criteria. Twenty-five families which fulfilled criteria for Li-Fraumeni-like (LFL, table 1) were tested including seven from the 21 identified in the current study.¹⁷ The families in this study fulfilling LFL criteria were compared with the 18 LFL families not including a single sarcoma or breast cancer occurrence.

Mutation detection was carried out as described previously.⁷ All exons (coding and non-coding), all splice junctions, the promoter, and the 3' untranslated region were analysed by direct sequencing. Any sequence variants were confirmed by sequencing the complementary strand. Wherever possible, multiple affected subjects from the families were analysed to verify that any putative mutation segregated with the disease. All these studies were carried out with approval of relevant local ethics committees.

Key points

- Mutations in the *TP53* gene account for the great majority (circa 70%) of families fulfilling classical criteria for Li-Fraumeni syndrome and a significant portion of families falling just short of these criteria.
- We have undertaken a study to determine the contribution of *TP53* germline mutations to families containing breast cancer and a single proven sarcoma, which fall short of classical criteria for LFS.
- Blood samples from a sarcoma patient or a first degree relative with breast cancer were analysed for mutations in *TP53* by direct sequencing of all exons, the promoter, and 3' untranslated region. Only one mutation was identified in 21 (5%) eligible families compared to 23/30 (77%) of families fulfilling classical Li-Fraumeni syndrome criteria.
- These results suggest that breast cancer on its own (in addition to a sarcoma) may not be a particularly strong marker for *TP53* mutations and that this should be taken into account in genetic counselling. The addition of even early onset breast cancer to a sarcoma may not be sufficient to justify *TP53* mutation testing.

Table 1 Diagnostic criteria for Li-Fraumeni syndrome and Li-Fraumeni-like syndrome

Li-Fraumeni syndrome ³	Li-Fraumeni like syndrome ¹⁷
Proband <45 years with a sarcoma	Proband with any childhood tumour, or sarcoma, brain tumour, or adrenocortical tumour <45 years
Plus 1st degree relative <45 years with any cancer	Plus 1st or 2nd degree relative in the same lineage with typical LFS tumour at any age or any cancer <45 years
Plus additional 1st or 2nd degree relative in the same lineage aged <45 years with any cancer or a sarcoma at any age	Plus another 1st or 2nd degree relative in the same lineage with any cancer <60 years

RESULTS

Only one mutation was identified in the 21 breast/sarcoma families studied. This family (family 2252, table 2) only failed to meet LFS criteria as the sarcoma was diagnosed four years after the qualifying date (49 rather than <45 years). There is also a suggestion of a further sarcoma in the mother of the tested subject who died from an intra-abdominal malignancy aged 23 years that has not been possible to confirm. The

Table 2 Cancers in eligible families

Family	LFL	Breast cancer	Osteosarcoma (age)	Other sarcoma	Other LFS spectrum cancers	Other cancers
729	Yes	42*, 52	Femur 9			
2063	Yes	47/49*, 58	Femur 13			
A	No	51*†	Femur 15	Ovary 59*†		
B	No	42*†, 32		48		Cervix 35*†
C	No	34*†		Abdomen 66*†		BCC 67*†
D	No	55*		Fibrosarcoma 67		Colon 42, RCC 39
E	No	55*†	Femur 18			Melanoma 31*†
F	No	35*†	Radius 40*†			
G	No	29*, 40†		Abdomen 45†		
H	No	31*		Uterus 62		
I	No	38*†	Femur 18*†			
J	No	42*, 62	Humerus 2			
K	No	45/54*†, 73		Synovial 27		Oesophagus 57*†
L	No	30, 44*†		Pelvis 55*†		
M	No	39*†		Fibrosarcoma 33*†		
N	No	37*†	Femur 15*†			
2252‡	Yes	25, 26/40*†		Leiomyosarcoma buttock 49*†	Glioblastoma 15, PNET 10, PNET 7	Abdominal malignancy 23
338	Yes	36/44*†		Chondrosarcoma 35*†		
80	Yes	35*†, 29, 60		Liposarcoma 30*		Ovary 51, rectal 44
328	Yes	45	16*		Glioma 56	
1799	Yes	37*†		Soft tissue 37*†		Stomach 48, 42

Cancers are those in the sarcoma patient and breast cancer patient and in their first degree relatives.

*Indicates subject tested. †Individual multiple primaries in a single person in a family (eg family 2252 contains a female with bilateral breast cancer aged 26 and 40 and a leiomyosarcoma aged 49 years); 45/50 indicates bilateral disease. ‡Indicates only family with proven *TP53* germline mutation. Numbered families are those classified as LFL, which are also previously published.

mutation, a 2 base pair deletion at codon 191 leading to a frameshift and a stop codon, has previously been reported by us,¹⁸ as have negative reports for the six other LFL families (table 2) with a case of a sarcoma and breast cancer (80, 328, 338, 348, 729, 2063).⁷ Family 348 was previously published as a LFL family but the breast cancer at 73 has been subsequently found to be DCIS. None of the other families has been reported previously.

We have now extended our survey of classical LFS families to 30 and detected mutations in 23 (77%). In LFL families, 10/25 (40%) had mutations. Excluding the seven LFL families in the current survey, mutations were detected in 9/18 (50%) compared to only 1/7 (14%) in the breast sarcoma set.

DISCUSSION

We have been rather surprised by the low rate of *TP53* mutations detected in families fulfilling our breast/sarcoma criteria. It is possible that we may not always have been able to test the most appropriate person (the sarcoma case) and that testing their affected mother may have failed to show a mutation that had occurred after conception (mosaic) but nonetheless was passed down to the affected offspring.¹⁹ However, this mechanism could only account for families A, L, and 338 as all other tested subjects in multi-case families were in the second or third generation of affected subjects (unless the first generation was a phenocopy). Nonetheless the four isolated breast/sarcoma double primaries (families C, E, I, and M) could also have been mosaic for a *TP53* mutation. It is unlikely that mosaicism would account for a significant miss rate in our study, as even in *NF2* the rate of mosaicism in de novo cases is no higher than 20%.²⁰ It is also possible that mutations in these families could have been missed owing to the sensitivity of the mutation techniques. However, given that mutations were detected in 77% of classical families, it is unlikely that more than one mutation would have escaped detection. It is possible that there may be another gene that accounts for Li-Fraumeni syndrome, which is more common in families with a predominance of breast cancer. However, recent evidence has shown that the *CHK2* gene may not be a real Li-Fraumeni syndrome gene.²¹ While an original report of one of our families with a *CHK2* mutation appeared to herald

this as the second LFS gene,²² it now appears that the mutation is a modifier of risk for genes other than *BRCA1* and *BRCA2* rather than a high risk allele in its own right.²¹

Although breast cancer is a very common feature in LFS and LFL families, it is probable that it is the presence of other characteristic tumours in addition to sarcoma that are the key predictors. It is of note that family 2252 (the only mutation positive LFL family) is the only one to contain at least two typical LFS tumours (PNET 10 years, glioblastoma 15 years) and that only two other families, 338 and 328, contained a single typical tumour with only one of these being childhood at onset. Three groups have now collectively analysed more than 800 unselected breast cancer patients for *TP53* germline mutations.²³⁻²⁵ Among these cases, germline *TP53* mutations were detected in only two (0.25%) so such mutations are clearly rare among apparently sporadic breast cancers. Two of these reports^{23, 25} along with a further report²⁶ have analysed series of breast cancers in patients selected because of family history of breast cancer or early onset (under 40 years of age) disease. A total of 274 such cases have been analysed and among these four patients with germline *TP53* mutations were detected (1.5%). We have recently analysed an even younger set of unselected patients aged 30 years or less and identified 4/99 (4%) with germline *TP53* mutations.²⁷ Two of these were predicted on the basis of a family history conforming to LFS or LFL and a further sporadic patient was shown to have a de novo change. It is clear, therefore, that germline *TP53* mutations account for only a small number even among selected breast cancer cases. Interestingly one of the LFL families in the <31 years set had a *BRCA2* mutation.²⁷

It is conceivable therefore that some of the families in table 2 could be caused by mutations in *BRCA1* or *BRCA2*. Half of them contain multiple cases of breast cancer and if sarcoma was a rare feature of *BRCA1/2* mutations it might not yet have been identified as such. Indeed, we have recently found an increased risk of sarcoma in the relatives of incident breast cancer cases.²⁸ Ignoring the sarcoma patients in each family, several of these might be expected to have mutations in *BRCA1/2*.

Perhaps the most surprising group of patients not to be identified with a *TP53* mutation were those with breast-sarcoma double primaries, in particular in family I where a

woman developed breast cancer at 38 years following an osteosarcoma at 18 years. However, Malkin *et al*²⁹ reported *TP53* germline mutations in only four of 59 children and young adults with second primary cancers and, indeed, this was later corrected to three out of the 59 (5%).

Chompret *et al*³⁰ have attempted to devise criteria to assess the sensitivity and positive predictive value of *TP53* germline mutation testing. Including their previous study of childhood tumours, they added a series of 116 breast cancers aged less than 36 years at diagnosis out of a series of 275 eligible cases. They identified three mutations in this series with two occurring in the context of classical LFS. One of these patients appeared to represent an isolated case of breast cancer at 31 years, but no information on testing of relatives was mentioned and this may have been *de novo*. Using the stringent criteria in this analysis, we have identified 5/21 families with an incident breast cancer <36 years in which a first or second degree relative developed an unquestioned LFS tumour (sarcoma, brain, breast cancer, ACC). Both the cases identified from the 116 incident breast cancer series had mutations if breast cancer was excluded as the cancer in the relative, but 0/21 had mutations if breast cancer was taken as the only relevant cancer. In contrast, we have identified only 1/5 (20%) of those fulfilling the stringent criteria in Chompret *et al*³⁰ (all our families fulfilled the breast cancer exclusion criteria as sarcoma was the main ascertainment criterion for our study). If we include the breast-sarcoma double primary cases (the sarcoma counted as a relative), this drops to 1/8 (12.5%) where the breast cancer was <36 years. Given that both the families in the French breast cancer series fulfilled LFS criteria, use of even the stringent criteria from this study is questionable. It would appear that LFS and LFL criteria have a much higher positive predictive value and specificity than the French stringent criteria. However, all these criteria will fall down on sensitivity given the possibility of *de novo* mutation.

In summary, our report has pointed to a low detection rate for *TP53* mutations in breast/sarcoma families not conforming to LFS. Indeed, it is questionable whether such testing should be initiated if the history does not even fulfil LFL criteria (LFL criteria excluding breast cancer as the main other tumour has a high positive predictive value, 50%). Certainly the 5% (1/21) mutation rate in the series as a whole is lower than the 10% guideline suggested by ASCO. Given the particularly difficult issues of genetic counselling and the low uptake of presymptomatic testing in *TP53* families,^{31 32} it is debatable how strongly the possibility of a *TP53* mutation should be raised, particularly in older onset breast/sarcoma families. While it is possible that such aggregations may be the result of other, as yet unidentified genes, the possibility that many of these could have occurred by chance should not be dismissed.

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