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). Sarcoma, particularly childhood osteosarcoma or rhabdomyosarcoma in addition to childhood adrenocortical carcinoma (ACC), is the strongest predictor of the presence of a TP53 mutation. However, while up to 80% of unselected series of ACC have TP53 germline mutations, only 3-10% of unselected sarcomas have been found to have such mutations. 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. While breast cancer is common in LFS and the penetrance of TP53 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), 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. 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. 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 breast cancer by the age of 50 in the general population in the western world. 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.

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
mutation, a 2 base pair deletion at codon 191 leading to a frameshift and a stop codon, has previously been reported by us, 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). 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. However, this mechanism could only account for those families with multiple cases of breast cancer and if sarcoma double primaries, in particular in family I where a single typical tumour with only one of these being childhood disease. A total of 274 such cases have been analysed and that only two other families, 338 and 328, contained a 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. 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 along with a further report 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. 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.

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. 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. 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. 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 a incident breast cancer <36 years in which a first or second degree relative developed an unquestoned 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 0/5 (20%) of those fulfilling the stringent criteria in Chompret et al. (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 0/8 (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 fully 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,11 12 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 another, as yet unidentified genes, the possibility that many of these could have occurred by chance should not be dismissed.

References