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Germline mutations of *TP53* and *BRCA2* genes in breast cancer/sarcoma families

Siranoush Manoukian[a], *Bernard Peissel*[a], *Valeria Pensotti*[b,c], *Monica Barile*[d], *Laura Cortesi*[e], *Silvia Stacchiotti*[f], *Monica Terenziani*[g], *Floriana Barbera*[b,c], *Graziella Pasquini*[h], *Simona Frigerio*[h], *Marco A. Pierotti*[c,h], *Paolo Radice*[b,c,*], *Gabriella Della-Torre*[h]

[a]*Medical Genetics Service, Department of Experimental Oncology and Laboratories, Istituto Nazionale Tumori, Milan, Italy*
[b]*Unit of Genetic Susceptibility to Cancer, Department of Experimental Oncology and Laboratories, Istituto Nazionale Tumori, Milan, Italy*
[c]*FIRC Institute of Molecular Oncology Foundation, Milan, Italy*
[d]*Division of Chemoprevention, European Institute of Oncology, Milan, Italy*
[e]*Department of Oncology and Haematology, University of Modena and Reggio Emilia, Modena, Italy*
[f]*Unit of Adult Sarcoma Medical Treatment, Department of Medical Oncology, Istituto Nazionale Tumori, Milan, Italy*
[g]*Unit of Paediatric Oncology, Department of Medical Oncology, Istituto Nazionale Tumori, Milan, Italy*
[h]*Unit of Molecular Mechanisms of Tumour Growth and Progression, Department of Experimental Oncology and Laboratories, Istituto Nazionale Tumori, Milan, Italy*

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ABSTRACT

The genetic aetiology of familial aggregations of breast cancer and sarcomas has been elucidated only in part. In this study, 23 unrelated individuals from families with one case of sarcoma and at least one case of breast cancer were screened for mutations in the *TP53*, *BRCA1* and *BRCA2* genes. Families were classified according to their conformity to the criteria defining the Li-Fraumeni syndrome (LFS), Li-Fraumeni-like (LFL) syndrome and hereditary breast/ovarian cancer (HBOC). Germline *TP53* mutations were identified in three instances (13%), including one LFS and two LFL families, while none of the non-LFS/non-LFL families had a *TP53* mutation. Three cases (13%), including one with a *TP53* mutation, carried *BRCA2* mutations. Of these, two were observed in LFL/HBOC families and the other one in a non-LFS/non-LFL/HBOC family, while none of the non-HBOC families tested positive. These findings suggest that the screening of *BRCA2*, in addition to *TP53*, may be appropriate for the molecular characterisation of breast cancer/sarcoma families, with practical implications for counselling and clinical management.

1. Introduction

Germline mutations of the *TP53* gene account for most families affected with the Li-Fraumeni syndrome (LFS),[1,2] an inherited condition characterised by the development of sarcomas and other early-onset tumours, including breast carcinoma.[3–6] In addition, they have been identified in a proportion of families with the Li-Fraumeni-like (LFL) syndrome, a phenotypic variant of LFS.[1,2,7] However, the genetic defects underlying familial aggregations with some LFS

* *Corresponding author:* Address: Unit of Genetic Susceptibility to Cancer, Department of Experimental Oncology and Laboratories, Istituto Nazionale Tumori, Milan, Italy. Tel.: +39 223903224; fax: +39 223902764.
E-mail address: paolo.radice@istitutotumori.mi.it (P. Radice).
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features, but failing to meet the LFS or LFL criteria, are not yet known. Recently, Evans and collaborators examined 21 families with a single sarcoma and one or more cases of breast cancer, not fulfilling the classical LFS criteria and found that only one of them (5%), a LFL family with clinical characteristics nearly coincident with LFS, had a *TP53* mutation.[7] This low mutation rate suggested that, outside LFS, the occurrence of breast cancer in association with a single sarcoma case is not a significant predictor of *TP53* mutations and that other genes might be involved in non-LFS breast cancer/sarcoma families.

BRCA1 and *BRCA2* are the main genetic risk factors for breast cancer known to date and are found to be mutated in families with the hereditary breast/ovarian cancer (HBOC) syndrome.[8] However, prior to this study their involvement in breast cancer/sarcoma families had not been investigated.

Here, we report on the analysis of 23 families with one case of sarcoma and at least one case of breast carcinoma, similar to those reported by Evans and colleagues,[7] that were screened for mutations in *TP53*, *BRCA1* and *BRCA2*, in order to asses the relative contribution of each gene to these families, in relation to the fulfilment of the clinical criteria for LFS, LFL and HBOC.

2. Patients and methods

Twenty families with a sarcoma and one or more breast cancer cases were identified from 1997 to 2004 during the genetic counselling activity at the Istituto Nazionale Tumori (INT), Milan. Three additional families with analogous phenotype were received from other Italian genetic counselling centres (European Institute of Oncology, Milan and Centre for the Familial Breast and Ovarian Cancer, Modena). Eligible cases were ascertained after an extensive interview for detailed family history with four-generation pedigree reconstruction performed during genetic counselling sessions. When possible, diagnoses of all reported cancers were verified by medical records. For each family one affected individual was selected for mutation screenings. Whenever possible (12 families), this was the case reporting the diagnosis of sarcoma. After signing a written informed consent to genetic investigations for research purposes, approved by the local ethics committees, enrolled cases provided blood samples for DNA screening.

Families were classified according to their conformity to LFS,[4] LFL[1] and HBOC criteria (Table 1). The latter were those in use at INT to select individuals eligible for testing in *BRCA* genes.

TP53 mutation detection was carried out by polymerase chain reaction (PCR) amplification and direct sequencing of all coding and non coding exons, all splice-site junctions, promoter and 3′ untranslated region. Mutation analysis of *BRCA1* and *BRCA2* genes was based on the use of denaturing high performance liquid chromatography (DHPLC), according to published protocols,[9,10] with minor modifications. This technique was employed to scan all coding exons and adjacent intronic sequences, with the exception of exon 11 of *BRCA1* and exon 11 of *BRCA2* that were examined by Protein Truncating Test (PTT), as previously described.[11] Samples showing altered patterns in DHPLC or truncated peptides in PTT were submitted to sequencing after PCR re-amplification.

3. Results

A description of the clinical and molecular data of the 23 subjects who underwent mutation screenings and of their families is reported in Table 2. Of the investigated families, two fulfilled the LFS criteria and five the LFL criteria. All LFS and LFL families and 13 out of the 16 non-LFS/non-LFL families matched the HBOC criteria.

Germline *TP53* mutations were identified in three families (13%). One mutation was observed in one (family no. 4) out of two LFS families and the others in two (nos. 2 and 7) out of 21 families (9.5%) failing to meet the stringent criteria of LFS. Both families were among those diagnosed as LFL, while none of the non-LFS/non-LFL families had a *TP53* mutation.

Table 1 – Diagnostic criteria for LFS, LFL amd HBOC

Li-Fraumeni syndrome
- Proband <45 years with a sarcoma
- plus 1st degree relative <45 years with any cancer
- plus additional 1st or 2nd degree relative in the same lineage aged <45 years with any cancer or a sarcoma at any age

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

Hereditary breast/ovarian cancer
- Female patients affected with breast cancer <36 years, or breast cancer plus ovarian cancer at any age, or male patients affected with breast cancer at any age, independently of family history
- Three or more first degree relatives[a] affected with breast cancer or ovarian cancer at any age
- Two first degree relatives[a] affected with :
  breast cancer <50 years
  breast cancer <50 years plus breast cancer bilateral at any age
  breast cancer <50 years plus ovarian cancer at any age
  breast cancer <50 years plus male breast cancer at any age
  ovarian cancer at any age

a or second degree relatives if in paternal lineage.

Table 2 – Clinical and molecular characteristics of breast cancer/sarcoma families analysed

Family no.	LFS/LFL criteria	HBOC criteria	Molecular analysis		Tested subjects			Other family members		
			TP53	*BRCA2*	Breast cancer	Sarcoma	Other cancers	Breast cancer	LFS spectrum cancers	Other cancers
1	LFS	Yes	wt	wt		**Chondrosarcoma, 19**		33, 41, 55†	Leukaemia, 81†	Abdomen, 50†
2	LFL	Yes	c.309C > A (p.Y103X)	wt		**Osteosarcoma, 17**		31		Stomach, 60–70
3	LFL	Yes	wt	wt	31				**Leiomyosarcoma, 39** Adrenocortical, (?)	
4	LFS	Yes	c.IVS4 + 1_IVS4 + 2insG	wt	34				**Liposarcoma, 44**	Ethmoidal, 42
5	LFL	Yes	wt	wt	46			48, 49, 49	**Osteosarcoma, 42** Brain (?), 62†	Colon, 32; Other (?), 68 Skin, 85
6	LFL	Yes	wt	c.6696_6697delTC	42			75 (male)	**Dermatofibrosarcoma, 30**	Larynx, 41
7	LFL	Yes	c.847C > T (p.R283C)	c.7408A > T (p.R2394X)	31, 66	Leiomyosarcoma, 71		60*	**Glioblastoma, 41** Astrocitoma, 25	Ovary, 39, 65*, 65
8	No	Yes	wt	wt		Rabdomyosarcoma, 15		55, 60, 66		Oesophagus, 61† Hodgkin's disease, 12
9	No	Yes	wt	wt	67				Osteosarcoma, 14	Ovary, 46, 53 Colon, 40, (?), (?)
10	No	Yes	wt	wt	53			56, 57*	Dermatofibrosarcoma, 55*	Pancreas, 84
11	No	Yes	wt	wt	29				Chondrosarcoma, 56	Stomach, 56 Pancreas, 32 Colon, (?)
12	No	Yes	wt	wt	34			38	Leiomyosarcoma, 49	Kidney, 44
13	No	Yes	wt	wt			Ovary, 74	37, 45, 46, 53, >50	Dermatofibrosarcoma, 48	
14	No	Yes	wt	wt			Ovary, 24	38	Leiomyosarcoma, 18 Brain (?), 30	
15	No	Yes	wt	c.9106C > T (p.Q2960X)	28, 31	Chondrosarcoma, 30		62		Ovary, 55; Melanoma, 44 Pancreas, 66 Stomach, 51, 53, 91 Colon, 72, 73
16	No	Yes	wt	wt	36	Osteosarcoma, 12		68*, 69*		
17	No	Yes	wt	wt	39	Rabdomyosarcoma, 38		44, 74		
18	No	Yes	wt	wt	24	Ewing sarcoma, 9				Stomach, 40† Bone (?), 37†
19	No	Yes	wt	wt		Myxoid sarcoma, 35†		46, 47		Lung, 75
20	No	Yes	wt	wt	76 (male)			67, 80, (?)	Leiomyosarcoma, 72	Lung, 60 Thyroid, 16
21	No	No	wt	wt	56	Leiomyosarcoma, 50				Lung (?), 50† Colon, 50
22	No	No	wt	wt	44	Sarcoma, 35		50, 68†		Stomach, 70 Other (?)
23	No	No	wt	wt	37	Angiosarcoma, 42		50		Lung, 76; Other (?)

Ages at tumour onset or at death (†) are indicated; (?) uncertain data; (*) tumour in the same individual.
Tested subjects of families no. 7, 22 and 23, received radiotherapy and developed sarcoma in the radiation field. Individuals identified as probands for clinical classification of LFS and LFL families are indicated in bold.

Identified alterations included a nonsense (Y103X), a splice site (IVS4 + 1_IVS4 + 2insG) and a missense (R283C) mutation. All mutations are most likely deleterious. The first is predicted to result in protein truncation and the second to influence mRNA splicing. Moreover, both the first and third mutation have been reported to occur as acquired alterations in sporadic tumours (p53 Soussi Mutation Database Cancer Website: http://p53.free.fr).

None of the tested subjects showed mutations in *BRCA1*, whereas three of them (13%), carried mutations in *BRCA2*. Two mutations were observed in LFL/HBOC families (nos. 6 and 7) and the other in one non-LFS/non-LFL/HBOC family (no. 15), while none of the non-LFS/non-LFL/non-HBOC families tested positive. Total *BRCA2* mutation rate in HBOC families was 15% (3/20).

Identified alterations included a frameshift (6696_6697delTC) and two nonsense (R2394X and Q2960X) mutations. All three mutations, which result in truncated proteins, had been already reported (http://www.nhgri.nih.gov/Intramural_research/Lab_tranfer/Bic).

Noticeably, in family no. 7 the tested subject carried both a *TP53* and a *BRCA2* mutation. The family history of this patient, who was diagnosed with bilateral breast cancer and leiomyosarcoma (see below), was singular as the phenotypic pattern in the maternal lineage was consistent with HBOC, while the presence of a brain tumour in one daughter and in the son of another daughter, who was asymptomatic, was diagnostic of LFL (Fig. 1). We could not perform molecular analyses in any affected relatives of the maternal lineage nor in the two individuals affected with brain tumour. However, the analysis of the asymptomatic daughter revealed the inheritance of a normal *TP53* allele and of the R2394X mutation in *BRCA2*.

Two of the *BRCA2* mutated subjects (from families no. 7 and 15) had developed a sarcoma as a second tumour following treatment for breast cancer. In one case, the *TP53*/*BRCA2* double mutant, a chest wall high grade leiomyosarcoma occurred 40 years after surgery and radiation therapy for the first breast tumour (in the radiation field). In the other one, a left humeral low grade chondrosarcoma occurred only two years after conservative surgery and radiation therapy for the first left breast cancer, but outside the field of irradiation.

In the third *BRCA2* mutated family, the tested subject was affected with breast cancer only, but we were able to confirm that also her brother who was diagnosed with a dermatofibrosarcoma, a low grade soft tissue sarcoma, carried the mutation. Thus, *BRCA2* mutations were overall identified in three subjects with a diagnosis of sarcoma.

No additional relative of mutated individuals was available for genetic testing.

4. Discussion

A significant excess of sarcomas in relatives of an unselected series of breast cancer patients has been recently reported by Bennett *et al.*, on the basis of follow-up data of more than 10 years.[12] This suggests the existence of common genetic risk factors for these malignancies. One obvious candidate is represented by the *TP53* gene, which is associated with LFS and LFL, two hereditary conditions predisposing to the development, among other tumours, of both sarcomas and breast carcinomas (reviewed in Ref. [13]). However, our analysis showed that in breast cancer/sarcoma families failing to meet the LFS classical definition *TP53* mutations are infrequent (2/21 = 9.5%) and are restricted to those with LFL phenotype. These findings confirm the very limited role of *TP53* gene previously demonstrated by Evans and collaborators in breast cancer/sarcoma families not consistent with LFS.[7] Accordingly, a few studies have reported that *TP53* mutations are rarely detectable in individuals

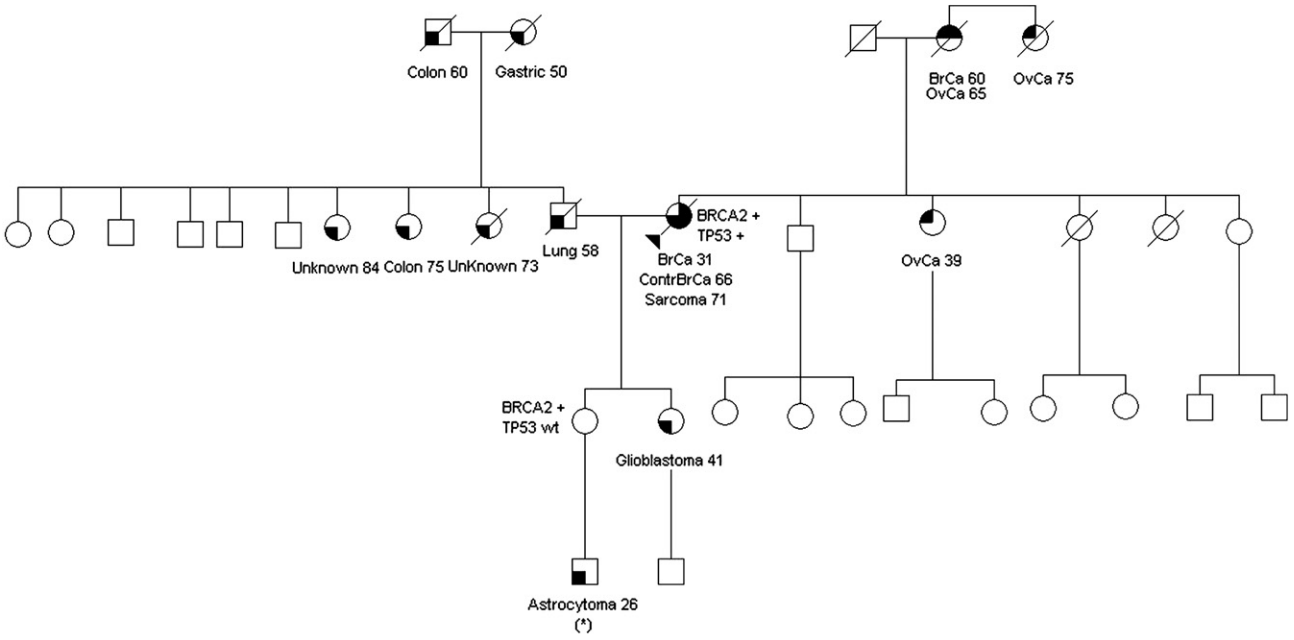

Fig. 1 – Pedigree of family no. 7. The affected subject analysed is indicated by arrow. (*) Paternal family history not suggestive of any hereditary tumour predisposition.

with evidence of hereditary susceptibility to breast cancer outside LFS and LFL families.[14–16]

We have also found that a fraction of breast cancer/sarcoma families, both LFL and non-LFS/non-LFL, which were consistent with the criteria that define HBOC, carried germline *BRCA2* mutations (3/20 = 15%). This value is in accordance with the total rate of *BRCA2* deleterious mutations (11%) we observed in 800 breast and/or ovarian cancer families screened at INT (unpublished). Sarcoma is a tumour outside the spectrum typifying HBOC and the association of sarcoma with germline mutations in BRCA genes has been reported only occasionally.[17] In this study, we ascertained the occurrence of *BRCA2* germline mutations in three patients affected with sarcomas, thus suggesting a possible role of the gene in the predisposition to this tumour. In favour of this hypothesis is the recent report of a possible increased risk for bone cancers in *BRCA2* mutation carriers,[18] albeit the authors of the study could not rule out the possibility that their observation was biased by the misdiagnosis of distant metastasis.

Contrary to what was observed for *BRCA2*, our series of breast cancer/sarcoma families lacked detectable *BRCA1* mutations. This difference might reflect the broader and more heterogeneous pattern of malignancies that is observed in association with *BRCA2* mutations, compared to *BRCA1* mutations.[18–20] However, the relatively small number of cases included in this study does not allow excluding that the difference observed could be due to chance. In fact, in an analysis of the etiologic heterogeneity of sarcomas, Lynch and collaborators reported a germline *BRCA1* mutation in a rabdomyosarcoma patient from a HBOC family.[17]

One intriguing finding of this study was the identification of an individual carrying both a *TP53* and a *BRCA2* mutation. Unfortunately, the lack of information on the segregation of each identified mutations among affected family members makes it difficult to establish their specific contribution to the observed family phenotype. In addition to bilateral breast cancer, the tested subject developed an apparently radiation-induced leiomyosarcoma that might be ascribed to the loss of *TP53* function.[13] However, in both her paternal lineages and siblings no LFS/LFL associated malignancy, except breast cancer, was reported, while the tumour pattern observed was clearly indicative of HBOC. A diagnosis of LFL was established based on the presence of brain tumours in two of her descendants, a daughter and a grandson, whose unaffected mother, however, inherited the *BRCA2*, but not the *TP53* mutation.

Although in our study *TP53* and *BRCA2* mutations accounted for only a minority of non-LFS breast cancer/sarcoma families, if confirmed, the observed mutation rates would provide justification for genetic testing of both genes for clinical purposes in these families. Moreover, whereas combining the observations of Evans and colleagues[7] with ours the screening for *TP53* mutations would seem appropriate for LFS and LFL cases only, testing for BRCA genes could contribute to a better understanding of the actual involvement of these genes in sarcoma risk.

Conflict of interest statement

None declared.

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