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BRCA1, BRCA2 and TP53 mutations in very early-onset breast cancer with associated risks to relatives

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ABSTRACT

Pathological mutations in *BRCA1*, *BRCA2* and *TP53* are associated with an increased risk of breast cancer. This study evaluated mutation frequency of these genes in early-onset breast cancer patients, and correlated this with family history and determined relative risks to family members. Patients with breast adenocarcinoma diagnosed ⩽30 years were ascertained between 1980 and 1997. Family history was established and mutation screening of *BRCA1*, *BRCA2* and *TP53* genes was performed. Estimates of penetrance and relative risk were undertaken. DNA was obtained from 100/139 women. 17/36 familial cases had a *BRCA1*, *BRCA2* or *TP53* mutation. Of 64 non-familial cases, one *BRCA2*, two *BRCA1* and two *TP53* mutations were detected. Penetrance estimates (by age 70) for breast cancer were 84% for *BRCA1* mutations and 91% for *BRCA2* mutations and for ovarian cancer, 60% and 26%, respectively. Relative risks associated with mutations were consistent with previous studies. *BRCA1* and *BRCA2* mutations in patients with breast cancer ⩽30 years are predicted strongly by family history. The majority of families with ovarian cancer were due to mutations in *BRCA1/2* whereas these mutations only accounted for 30–50% of the excess breast cancers.

1. Introduction

A proportion of early-onset and familial breast cancer is due to germline mutations in three genes, *BRCA1*, *BRCA2* and *TP53*. These mutations confer a lifetime breast cancer risk of 43–85%.[1,2] Several studies have investigated the frequency of *BRCA1/2* mutations in families with breast and/or ovarian cancer[3] and of *TP53* mutations in Li Fraumeni (LFS) or Li Fraumeni Like (LFL) syndrome.[4] Amongst breast cancer cases unselected for family history, the prevalence of *BRCA1/2*

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mutations is dependent on the population studied and the age at diagnosis of malignancy.[5–8] Whilst several authors have determined the proportion of *BRCA1/2* and *TP53* mutations in population-based series of breast cancer cases[6–9] these only studied small numbers diagnosed with breast cancer at ⩽30 years of age, and none undertook mutation screening of all three genes. In addition, the value of a full family history in identifying mutation carriers was not clarified.

The aim of this study was to estimate the contribution of *BRCA1*, *BRCA2* and *TP53* to early-onset breast cancer (⩽30 years) and to explore the utility of family history in identifying mutation carriers. The detection frequency and penetrance estimates from this study have been published in a short research letter.[10] This paper presents the detailed analysis of the relative risk of breast/ovarian cancer in first-degree relatives in these families both with and without *BRCA1/2* mutations. It also presents results of analysis from an extended series of families with an index case diagnosed with breast cancer at ⩽30 years of age.

2. Patients and methods

2.1. Patients

Patients were ascertained from the North Western Cancer Registry (NWCR) covering a population of 4.1 million in North Western England, with a diagnosis of breast cancer at or under 30 years of age between 01/01/1980 and 31/12/1997. Diagnoses were confirmed using hospital records and pathology reports. Those patients with a histological diagnosis other than breast carcinoma were excluded. Patients were approached via their consultant for permission to be interviewed and, after informed consent, to provide blood for DNA testing. A detailed three-generation pedigree was obtained along with copies of hospital notes where available. Any record within hospital records regarding family history at initial presentation or diagnosis of malignancy was noted. This was compared to the ascertained family history of malignancy at that point in time. Family histories of malignancy were confirmed via the cancer registry or death certificates.

Patients were categorised into familial, Li–Fraumeni syndrome (LFS), Li–Fraumeni like syndrome (LFL) or non-familial groups based on family history at initial diagnosis. Familial patients were defined as those with a family history of breast cancer <65 years or ovarian cancer at any age in first or second-degree relatives. LFS and LFL were defined as in Table 1. Patients diagnosed after January 1993 were approached on a semi-prospective basis.

A further series of families, identified through the North West Regional Genetics Service, with at least one relative diagnosed with breast cancer at ⩽30 years also underwent screening for mutations in *BRCA1/2*.

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

Li–Fraumeni syndrome[25]	Li–Fraumeni like syndrome[26]
Proband <45 years with a sarcoma	Proband <45 years with childhood tumour, sarcoma, brain tumour or adrenocortical tumour
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
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

2.2. Mutation analysis

All samples were screened for mutations in *BRCA1*, using the protein truncation test (PTT) for exon 11 and single strand conformation polymorphism/heteroduplex analysis (SSCP/HA) for the remaining exons.[11,12] *BRCA2* was analysed using the same approach for the familial cases. Non-familial cases were screened by fluorescent chemical cleavage of mismatch (FCCM) analysis with hydroxylamine hydrochloride as previously described.[13] This method has been reported to detect >95% of mutations[14]; however as only hydroxylamine hydrochloride (which detects mismatched cytosine bases) was used, this method would not be expected to detect A > T or T > A transversion mutations. Sequence variants were confirmed by direct sequencing. All mutations detected by SSCP/PTT were confirmed by FCCM to confirm the sensitivity of this technique. Further mutation screening of *BRCA1* and *BRCA2* has been undertaken using Multiple Ligation-dependent Probe assay (MLPA).

All 10 coding exons, splice junctions and the promoter region of *TP53* were analysed by direct sequencing in all cases.[4]

2.3. Penetrance analysis

Pedigree information for mutation carriers was used to estimate the cumulative cancer risks in *BRCA1* and *BRCA2* mutation carriers using the programme MENDEL.[15] The pedigree information was updated to the last point of contact with the family. Since ascertainment of the families was on the basis of a single affected index case the conditional likelihood of the pedigree was maximised given the phenotype and genotype of the index case at ascertainment, i.e., $L(\text{pedigree}|\text{index}) = L(\text{pedigree})/L(\text{index})$.

We parameterised the model in terms of log relative risk for breast and ovarian cancer in mutation carriers compared to population risks for the United Kingdom. Non-gene carriers were assumed to develop the disease at population incidence rates. The breast cancer relative risks (RR) were allowed to vary with age using three age groups; 20–39, 40–59, and 60–79. The relative risk of ovarian cancer was assumed to remain constant with age. The cumulative risks or penetrance were calculated from the cumulative incidence $\Lambda(t)$, where:

$$\Lambda(t) = \sum_{k=1}^{n} i_k t_k \exp(\beta_k)$$

where i_k is the incidence of the kth age band of length T_k and $\beta_k = \ln(\text{RR})$ in kth age band. The cumulative risk $F(t)$ is then given by $F(t) = 1 - \exp(-\Lambda(t))$.

The associated confidence limits are given by:

$$F(t) = 1 - \exp(-\lambda(t) \pm 1.96\sqrt{\text{Variance}\,\lambda(t)})$$

where

$$\text{Variance}\,\lambda(t) = \sum_{k=1}^{n} i_k t_k^2 \,\text{var}(\beta_k)\,\exp(2\beta_k) + 2\sum_{\substack{j<k \\ k=1}}^{n} i_k i_j T_k T_j [\text{var}(\beta_k)\text{var}(\beta_j)]^{1/2} \times \exp(\beta_k)\exp(\beta_j)\text{corr}(\beta_k, \beta_j)$$

2.4. Risk in relatives

The relative risks for breast or ovarian cancer for first-degree relatives of the patients identified from the NWCR database were calculated for the period 1979–1998. As a basis for calculating the expected number of cancers, population-based incidence rates for breast and ovarian tumours for each of the years 1979–1998 were obtained from the NWCR. Person-years-at risk was measured from 1.1.1979 to whichever of these occurred first: 31.12.1998, date of interview of index case or date of death of relative. Age group and calendar-year specific person-years at risk were multiplied by the corresponding incidence rates to produce the number of cancers that would have been expected to occur assuming that general population incidence rates applied. The relative risk was estimated by dividing the number of cases observed by the expected number.

3. Results

Two-hundred and seventy-eight women resident in the area covered by the NWCR were identified on the Registry database with early-onset primary breast cancer diagnosed between 1/1/80 and 31/12/97. At ascertainment, 117(42%) women were dead, 137(49%) were living. Information was unavailable on 24(9%) cases. The age of diagnosis and grade of tumour did not significantly differ between the living and dead cases. The mean age at diagnosis was 28 years 3 months (range 19 years 5 months–30 years 11 months). Consultant permission to approach the patient was refused for 7 living patients. Of the remaining 130 cases, 91 consented to participate, 35 refused and 4 could not be traced. Blood samples were obtained from 66% of living affected women and 33% of the whole series.

A further 23 women registered with the NWCR were living outside the regional boundaries. They were registered on the NWCR as they had been treated at hospitals within the region. Nine agreed to take part in the study. In total, therefore, 100 samples were analysed for mutations in *BRCA1*, *BRCA2* and *TP53*.

Thirty-seven patients (37%) had a significant family history, which was consistent with LFS in 2 cases and LFL in 3 cases. The remaining 63 cases were non-familial.

3.1. Molecular analysis

Overall, mutations in *BRCA1*, *BRCA2* and *TP53* were identified in 18/37 (49%) familial cases and 4/63 (6%) of the non-familial cases.

3.2. BRCA1/2 analysis

Pathogenic *BRCA1* mutations were identified in 10/100 women (10%) diagnosed with breast cancer at ⩽30 years. *BRCA2* mutations were detected in 8 women (8%) (Table 2, cases 1–18). A further 5 mutations of unknown significance were also demonstrated (Table 3).

(a) Familial cases:
Family history was a strong predictor of mutation status; 8/10 *BRCA1* mutation carriers and 7/8 *BRCA2* mutation carriers had a positive family history. In case 7, the ovarian cancer was diagnosed in her mother after the diagnosis of the index patient but before the study cut-off point. One mutation in *BRCA2* was identified after the original study by MLPA analysis (Table 2, case 18). In summary, mutations in *BRCA1/2* were demonstrated in 15/37 (41%) familial cases.

(b) Non-familial cases:
Three non-familial cases (3/63) had pathogenic mutations, two in *BRCA1* and one in *BRCA2*. Case 5 had a paternal grandmother who developed breast cancer at the age of 66 years and was therefore classified as a non-familial case. This family were Ashkenazi Jews with the 185delG mutation in *BRCA1*. The second *BRCA1* mutation was demonstrated on MLPA after the original study. This patient had developed bilateral medullary breast cancer at ages 30 and 41 years and had little information on her relatives from Eastern Europe (case 10). Case 14 was classified as a non-familial case and was shown to have a *BRCA2* mutation. There was a history of early-onset breast cancer in a third-degree relative and her mother (a mutation carrier) has subsequently developed an endometrial malignancy.

3.3. TP53 analysis

In total, 4/100 (4%) mutations were demonstrated in *TP53*.

(a) Familial cases:
Amongst familial cases 2/37 (5%) mutations were isolated in the *TP53* gene. Both were identified within families that fulfil LFS/LFL criteria, one in each group. Neither family history had been documented at diagnosis in the medical notes, although the family history had been significant at that point in time.

(b) Non-familial cases:
2/63 (3%) of the non-familial group had identifiable mutations in *TP53*: Case 22 had a mutation at codon 273 causing an amino-acid substitution of histidine for arginine. This woman developed high-grade comedo DCIS aged 28 years and recurrence of comedo DCIS with several areas of invasive ductal carcinoma aged 30 years. Aged 31 years she developed a clear-cell renal carcinoma and at 34 years of age, a sarcoma behind the functional kidney that had been examined with numerous intravenous urograms with

Table 2 – Pathogenic mutations in BRCA1/2 and TP53

Patient	Gene	Mutation	FH breast cancer	FH ovarian cancer
Case 1	BRCA1	185delAG	Mother 53	Nil
Case 2	BRCA1	153C > T	Mother, aunt	Nil
Case 3	BRCA1	2682C > T	Mother bilat 32/38, mat gm 70	Nil
Case 4	BRCA1	4182delAATC	Mother 43, mat cousin 34	Mat aunt 46
Case 5	BRCA1	185delAG	Pat gm 66	Nil
Case 6	BRCA1	3477delGT	Nil	Mother 43, mat aunt 50
Case 7	BRCA1	4744delT	Nil	Mother 68[a]
Case 8	BRCA1	IVS6 421-2DelA	Sister bilateral 34/37, mother 44, 2 mat aunts 48, 60 mat cousin 28	Nil
Case 9	BRCA1	1875delC	Mother 41, mat gm 68	Mother 41, mat aunt 43, mat gt aunt 45
Case 10	BRCA1	Duplication exons 18 and 19	Nil	Nil
Case 11	BRCA2	6819delTG	26 Relatives	Nil
Case 12	BRCA2	9132delC	Pat gm 59	Nil
Case 13	BRCA2	2157delG	Nil	Mother 48
Case 14	BRCA2	3826delTC	Nil	Nil
Case 15	BRCA2	6503delTTT	Mat gm 40	Nil
Case 16	BRCA2	6503delTT	Mother 50	Mother 58[a]
Case 17	BRCA2	800delAT	Mother 61	Pat gm 71
Case 18	BRCA2	Deletion exon 2	Mother 29, mat gmother 52, mat gaunt 65, mat gaunt 75, mat gt gmother 50	Nil
Case 19	TP53	273G > A	Sister	Nil
Case 20	TP53	366 T > G	Nil	Nil
Case 21	TP53	191delCC	Mother	Nil
Case 22	TP53	273 G > A	Nil	Nil
FH1	BRCA1	5622C > T	Pat aunt 38, pat gt aunt 40, pat gmother 38	Nil
FH2	BRCA1	2073delA	Mother 42, Sister 50/52	Mother 52, sister 46, sister 50
FH3	BRCA1	5362G > A	Mat aunt 44, mat gt aunt 55, cousin 26	Mother 46, mat gmother 43
FH4	BRCA1	4184del4	Mother 69, mat aunt 43	Mat aunt 49
FH5	BRCA1	185delAG	Sister bilat 41/57, niece 44, gt niece 41	Nil
FH6	BRCA1	188del11	Daughter 56, grand daughter 50	Nil
FH7	BRCA1	5271 + 4 A > G	Mother bilat 32/38, 6 others	Mt gt aunt 68
FH8	BRCA1	2799delAA	Daughter 31, pat aunt 54, pat cousin 36	Sister 45, sister 55
FH9	BRCA1	Exon 13 dup	Mother 51, mat gm 62, mat aunt 58, mat cousin 48	Mat aunt 62
FH10	BRCA1	185delAG	Mother 48, mat gmother, mat cousin 45, mat niece 47	Nil
FH11	BRCA1	4184del4	Mother 42, mat gt aunt 45	Nil
FH12	BRCA2	2157delG	Mother 32, mat aunt bilat 56, mat aunt 41, mat gfather 68	Nil
FH13	BRCA2	7771 insA	Mother bilat 42/49, mat gm bilat 33/57	Nil
FH14	BRCA2	1418 ins TTAG	Mother 40, sister 34	Nil
FH15	BRCA2	S2984X	Niece 32, niece 34, niece 45, niece 33	Nil
FH16	BRCA2	9318 insA	Brother 40, mother 62, pat aunt bilat 36, pat cousin bilat 39	Gt aunt 60
FH17	BRCA2	6503delTT	Daughter bilat 48, brother 38	Nil

Table 2 – *continued*

Patient	Gene	Mutation	FH breast cancer	FH ovarian cancer
FH18	*BRCA2*	5910C > G	Self bilat 29/48, mother 37, mat aunt 42, mat gm 50, mat cousin 55	Nil
FH19	*BRCA2*	3034delAAAC	Sister 39, sister 47, niece 56	Nil
FH20	*BRCA2*	2157delG	Mother 56, mat aunt 44, cousin 35	Mat cousin 47

Cases – original cohort of women ascertained via the cancer registry; FH – expanded cohort of families with a case diagnosed at 30 years or younger; mat – maternal, pat – paternal; gm – grandmother.
a Ovarian cancer occurred after diagnosis of index case.

Table 3 – Unknown variants found in BRCA1 and BRCA2

Patient	Gene	Unknown variant	FH of breast cancer	FH of ovarian cancer
Case 23	*BRCA1*	Q804H	Nil	Nil
Case 24	*BRCA2*	2344G to A	Pat gm 70	Nil
Case 25	*BRCA2*	451G to C	Pat aunt 60, pat aunt 32	Nil
Case 26	*BRCA2*	7049G to T	Nil	Nil
Case 27	*BRCA2*	9485-16T to C	Pat aunt 60	Nil

the resultant exposure to radiation. Both parents were unaffected at age 58 years and do not carry the mutation.

A missense mutation, 366 serine to alanine, was described in case 20. This was present in the proband's mother who was diagnosed with transitional cell carcinoma of the bladder at 61 years. Whilst this mutation is previously undescribed, it is likely to be pathogenic. 366Ser is a conserved residue amongst mammals, and the mutation is inherited from an affected parent.

3.3.1. *Mutation detection frequencies with different family histories*

Table 4 presents family history data of the NWCR cases and an additional 54 families referred through to the North West regional genetics service in which an individual aged ⩽30 years had been diagnosed with breast cancer. Fifty percent of families with a history of breast cancer <60 years or ovarian cancer at any age as well as the index case, had a detectable pathogenic mutation in *BRCA1/2*. As expected, a family history of ovarian cancer was more significant. The detection rate with a family history of only breast cancer was 20% as compared to 75% if the family history included a case of ovarian cancer. 3/3 families with only a single case of ovarian cancer as well as the index case had pathogenic mutations.

3.4. *Penetrance analysis*

The cumulative risks of breast and ovarian cancer in carriers of a pathogenic mutation in *BRCA1* or *BRCA2* are given in Table 5. These figures are calculated from those families in the population-based study ($n = 91$).

Table 4 – Mutation detection frequencies in BRCA1/2 in all families (100 NWCR and a further 54 families) tested containing a proven case of breast cancer aged 30 years or younger

Cancers in relatives	*BRCA1*	*BRCA2*	No mutation	Total
Sporadic (none)	0	1 (1.5%)	67	1/68 (1.5%)
Bilateral in index case nil in relatives	1	0	3	1/4 (25%)
Breast cancer >60	1[a] (11%)	0	8	1/9[a] (11%)
1 Breast cancer <60	2 (13%)	1 (7%)	12	3/15 (20%)
2 Breast cancer <60	3	2	8	5/13 (38%)
3 Breast cancer <60	1	2	6	3/9 (33%)
4+ Breast cancer <60	4	3	2	7/9 (78%)
Ovarian cancer	10 (50%)	5 (25%)	5	15/20 (75%)
Male breast cancer	0	4 (57%)	3	4/7 (57%)
Any MBC, ovary or breast <60	21	17	36	38/74 (51%)
Total	22/154	18/154	114	40/154

Definition by cancers in relatives.
a Ashkenazi Jewish family.

Table 5 – Cumulative risks of breast and ovarian cancer with pathogenic mutations in BRCA1 and BRCA2

	Cumulative risk (%) breast cancer (95% CI)		Cumulative risk (%) ovarian cancer (95% CI)	
	BRCA1	*BRCA2*	*BRCA1*	*BRCA2*
40	17 (0–33)	24 (0–45)	0	0
50	49 (16–69)	61 (19–81)	26 (0–47)	14 (0–37)
60	58 (19–79)	84 (25–97)	39 (0–64)	15 (0–37)
70	87 (22–98)	95 (30–100)	61 (0–88)	15 (0–38)

Table 6 – Relative risks of breast and ovarian cancer

All cases (99)	Mothers (94)		Sisters (119)		Total (213)	
	Number of tumours	RR	Number of tumours	RR	Number of tumours	RR
Breast cancer	16	6.1	3	7.6	19	6.3
Ovarian cancer	2	4.2	1	11.8	3	5.3

One sister had both breast and ovarian cancer and has been counted separately for both.

Table 7 – Relative risk of breast cancer within these families by mutation status[a]

	Relative risk
BRCA1 or *BRCA2* pathogenic mutation present ($n = 18$)	26.2
BRCA1 or *BRCA2* pathogenic mutation absent ($n = 72$)	3.9

a Families whose index case had a *TP53* mutation or a *BRCA* mutation of unknown pathogenicity have been excluded from this analysis.

3.4.1. *Relative risk analysis*

Data from a person years at risk analysis for mothers and sisters of the index cases are presented in Table 6. Cancer incidence is compared to the rates for the population covered by the NWCR. The risk of both breast and ovarian cancer are substantially increased at around 6-fold. Although the majority of the excess risk is due to *BRCA1/2* mutations, with mutation positive families having an increased risk of breast cancer of 20-fold, there is still an increased risk in the remaining families (Table 7).

4. Discussion

This is the first comprehensive analysis of family history and mutation analysis in all three high-risk breast cancer genes in women diagnosed with breast cancer aged 30 years or less.The prevalence of *BRCA1/2* and *TP53* mutations in this cohort has been previously published.[10]

The mutation detection rate in familial breast cancer in these three genes was almost 50%, demonstrating the importance of accurately documenting a family history. Only 55% of cases had documentation in the notes on admission for investigation of a breast lump and much of this family history was inaccurate.

The overall number of DNA samples obtained from the cohort was only 33%, which demonstrates the limitations of a retrospective study. From 1993 the women were ascertained on a semi-prospective basis. However, as there was no difference in the proportion of familial and sporadic groups pre and post-January 1993, it is unlikely that this biased the results. It could be suggested that the percentage of mutation carriers in the deceased population is higher due to the aggressive nature of breast cancer associated with *BRCA1* mutations. However, these women are likely to have a family history and the results of the study would therefore remain consistent.

Most *BRCA1* and *BRCA2* mutation carriers had at least one first or second-degree relative with breast or ovarian cancer further suggesting that family history is a reliable indicator for the presence of a pathogenic mutation in *BRCA1* or *BRCA2*. In addition, few mutations were found in those women without a family history. These data would therefore not support testing of *BRCA1/2* in patients without a family history of breast cancer. These results are consistent with data from Loman and colleagues,[7] but in contrast with the findings of Peto and colleagues[6] who identified mutations in several patients in a population based study that had reported no family history in first-degree relatives. However, in the latter study, a complete family history to include all second-degree relatives was not elicited. This may exclude paternal family history. A study from Spain[16] suggested that relying on family history alone would miss a considerable proportion of mutations in *BRCA1* and *BRCA2*. This study however, only included a minority of cases diagnosed under the age of 30 years and had a more stringent definition of family history. Applying our criteria for family history to the Spanish study, 7/9 of their mutation carriers had a family history.

The likelihood of mutation detection in *BRCA1/2* is increased in families with at least one relative diagnosed less than 30 years. In comparison to the breast cancer linkage consortium (BCLC) data, where *BRCA1/2* mutations only accounted for about 50% of families with 4 or more cases of breast cancer only[17,18] this series identified mutations in

78% of such families. It is unlikely that the BCLC series contained many early-onset cases.

This is the first estimation of the incidence and prevalence of *TP53* mutations and suggests that the frequency of mutations is higher that expected. The three pathogenic mutations and one probable mutation in this series accounts for 4% of breast cancer ⩽30 years and the majority of patients with second primaries outside the breast. Previous reports had suggested that *TP53* is an infrequent cause of familial breast cancer and accounts for <1% of breast cancer <40 years.[19] However, the very high relative risk of breast cancer <31 years (RR > 100) (Chompret and colleagues) means that *TP53* should be considered in very young breast cancer patients as it may impact upon subsequent management. For example, within the North West, the management of a 20-year-old patient presenting with breast cancer was altered from a wide local excision and radiotherapy to mastectomy following discovery of a family history consistent with LFS and subsequent detection of a germline *TP53* mutation. Excess radiation should be avoided in *TP53* mutation carriers as demonstrated by the case with a sarcoma in the field of radiation from regular IVUs.

The diagnosis of a second primary tumour should also prompt the clinician to consider a *TP53* mutation. Renal cell carcinoma is not normally considered a feature of LFS[4] but has been previously reported in a mutation carrier.[20] This patient is the only de novo *TP53* mutation that we have proven although de novo mutations in *TP53* are relatively common.[21]

The penetrance estimates from this study are similar to BCLC data,[1] but higher than those reported by other population based studies.[22,23] However, they are consistent with the most recent meta-analysis of population studies.[24] Whilst the confidence intervals on all these estimates are wide, a possible explanation for differences with other population-based studies is that the very early age of onset of breast cancer in our study may be due to the presence of genetic modifiers of risk. Given the small numbers involved in this study, it would be more appropriate when counselling families to base risk estimates on the figures from the meta-analysis.[24]

The ovarian cancer risk in this cohort of patients is also higher than in previous estimates. All those familial cases with a family history of ovarian cancer were found to have a mutation in *BRCA1* or *BRCA2* in the population series and 75% (15/20) in the extended series. These mutations therefore account for all the excess ovarian cancer within this cohort of early-onset breast cancer patients and almost certainly in families presenting with ovarian cancer and breast cancer ⩽30 years in genetics clinics.

The relative risk estimates for this cohort with mutations in *BRCA1/2* is comparable with previous studies. The increase in relative risk of breast cancer in those families without mutations is consistent with other genes involved in familial breast cancer.

In summary, this study demonstrates the high likelihood of mutations in *BRCA1/2* and *TP53* in women with early-onset breast cancer in the context of a family history.

Ascertaining this family history should therefore be an important part of the assessment of women with breast cancer diagnosed at the age of 30 years or younger. It will allow appropriate targeting of mutation testing and therefore management of that individual and potentially the wider family.

Conflict of interest statement

None declared.

REFERENCES

1. Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the *BRCA1* and *BRCA2* genes in breast cancer families. *Am J Hum Genet* 1998;**62**:676–89.
2. Li FP, Fraumeni JF. Prospective study of a cancer family syndrome. *JAMA* 1982;**247**:2692–4.
3. Gayther SA, Pharoah PDP, Ponder BAJ. The genetics of inherited breast cancer. *J Mamm Gland Biol Neopasia* 1998;**3**:365–76.
4. Varley J, McGown G, Thorncroft M, et al. Germ-line mutations of TP53 in Li–Fraumeni families: an extended study of 39 families. *Cancer Res* 1997;**14**:865–71.
5. Fitzgerald MG, Macdonald DJ, Krainer M, et al. Germ-line *BRCA1* mutations in jewish and non-jewish women with early-onset breast cancer. *N Engl J Med* 1996;**334**:143–9.
6. Peto J, Collins N, Barfoot R, et al. Prevalence of *BRCA1* and *BRCA2* gene mutations in patients with early onset breast cancer. *J Natl Cancer Inst* 1999;**91**:943–9.
7. Loman N, Johannsson O, Kristoffersson U, Olsson H, Borg A. Family history of breast and ovarian cancers and *BRCA1* and *BRCA2* mutations in a population-based series of early onset breast cancer. *J Natl Cancer Inst* 2001;**93**:1215–23.
8. Krainer M, Silva-Arrieta S, Fitzgerald MG, et al. Differential contributions of *BRCA1* and *BRCA2* to early onset breast cancer. *N Engl J Med* 1997;**336**:1416–21.
9. Sidransky D, Tokino T, Helzlsouer K, et al. Inherited *p53* gene mutations in breast cancer. *Cancer Res* 1992;**52**:2984–6.
10. Lalloo F, Varley J, Ellis D, et al. Prediction of pathogenic mutations in patients with early-onset breast cancer by family history. *Lancet* 2003;**361**:1101–2.
11. van Der Luijt R, Khan PM, Vasen H, et al. Rapid detection of translation-terminating mutations at the adenomatous polyposis coli (APC) gene by direct protein truncation test. *Genomics* 1994;**20**:1u–4u.
12. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci USA* 1989;**86**:2766–70.
13. Ellis D, Greenman J, Hodgson S, et al. Low prevalence of germline *BRCA1* mutations in early onset sporadic breast cancer. *J Med Genet* 2000;**37**:792–4.
14. Rowley G, Saad S, Gianelli F, Green P. Ultrarapid mutation detection by muliplex, solidphase chemical cleavage. *Genomics* 1995;**30**:574–82.
15. Lange K, Weeks D, Boehnke M. Programs for Pedigree Analysis: MENDEL, FISHER, and dGENE. *Genet Epidemiol* 1988;**5**:471–2.
16. de Sanjose S, Leone M, Berez V, et al. Prevalence of *BRCA1* and *BRCA2* germline mutations in young breast cancer patients: a population-based study. *Int J Cancer* 2003;**106**:588–93.
17. Easton DF, Bishop DT, Ford D, Crockford GP. Breast cancer linkage consortium. Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. *Am J Hum Genet* 1993;**52**:678–701.

18. Narod SA, Ford D, Deville P, et al. An evaluation of genetic heterogeneity in 145 breast–ovarian cancer families. *Am J Hum Genet* 1995;**56**:254–64.
19. Börresen AL, Anderson TI, Garber J, et al. Screening for germline TP53 mutations in breast cancer patients. *Cancer Res* 1992;**52**:3234–6.
20. Sedlacek Z, Kodet R, Kriz V, et al. Two Li–Fraumeni syndrome families with novel germline p53 mutations: loss of the wild-type p53 allele in only 50% of tumours. *Br J Cancer* 1998;**77**:1034–9.
21. Chompret A, Brugieres L, Ronsin M, et al. P53 germline mutations in childhood cancers and cancer risk for carrier individuals. *Br J Cancer* 2000;**82**:1932–7.
22. Anglian Breast Cancer Study Group. Prevalence and penetrance of *BRCA1* and *BRCA2* in a population based series of breast cancer cases. *Br J Cancer* 2000;**83**:1301–8.
23. Hopper JL, Southey MC, Dite GS, et al. Population based estimate of the average age-specific cumulative risk of breast cancer for a defined set of protein-truncating mutations in *BRCA1* and *BRCA2*. Australian Breast Cancer Family Study. *Cancer Epidemiol Biomarkers Prev* 1999;**8**:741–7.
24. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with *BRCA1* or *BRCA2* mutations detected in case series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet* 2003;**72**:1117–30.
25. Li FP, Fraumeni JF, Mulvihill JJ, et al. A cancer family syndrome in twenty-four kindreds. *Cancer Res* 1988;**48**:5358–62.
26. Birch JM, Hartley AL, Tricker KJ, et al. Prevalence and diversity of constitutional mutations in the p53 gene among 21 Li–Fraumeni families. *Cancer Res* 1994;**54**:1298–304.