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Incidence of *BRCA1/2* germ line alterations in a high risk cohort participating in a phase II chemoprevention trial

J. Klemp [a], D. Brady [a], T.S. Frank [b], B.F. Kimler [c], C.J. Fabian [c,*]

[a] *Division of Clinical Oncology, Department of Internal Medicine, University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City, KS 66160-7418, USA*

[b] *Myriad Genetic Laboratories, Inc., 320 Wakara Way, Salt Lake City, UT 84108-9930, USA*

[c] *Department of Radiation Oncology, University of Kansas Medical Center, Kansas City, KS 66160-7820, USA*

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Abstract

It is unknown what proportion of women at high risk for breast cancer, entering phase II chemoprevention trials, have *BRCA1/2* alterations, and whether their initial biomarker patterns or response to preventive interventions will differ between carriers and non-carriers. As part of a 6-month phase II chemoprevention trial of diflouromethlyornithine (DFMO), high-risk subjects (family history, prior precancerous breast disease or prior breast cancer), who had random peri-areolar fine needle evidence of epithelial hyperplasia with or without atypia, were offered genetic counselling and testing at the completion of their study participation. 97% of the 119 women eligible for testing underwent *BRCA1/2* gene sequencing, 3 declined. 26 (22%) of the 116 women had an alteration in *BRCA1/2*. Known deleterious mutations were present in 3 (3%), uncertain significance mutations in 19 (16%), and probable polymorphisms in 6 (5%). There does not appear to be a difference in initial biomarker distribution between participants with and without germ line alterations.

Keywords: *BRCA* mutation; Prevention trial

1. Introduction

Although less than 1% of the general population is estimated to harbour a deleterious mutation in *BRCA1* or *BRCA2* [1], women who carry such a mutation have a 56–87% lifetime risk of developing breast cancer [2,3]. Women from families with an identified *BRCA1/2* mutation are likely to be interested in preventive strategies due to the high likelihood of cancer development and may opt to participate in high risk clinics and prevention trials. Preventive interventions for gene carriers include careful surveillance, prophylactic mastectomy, prophylactic oophorectomy and chemoprevention [4–8].

Recent studies indicate that breast cancers arising in women with a *BRCA1* mutation are more likely to exhibit higher nuclear grade, lack oestrogen and progesterone receptor expression, overexpress p53 and exhibit higher proliferation rates than cancers arising in non-mutation carriers [9–12]. Tumours from women with *BRCA2* mutations generally are low to intermediate grade [13] and the majority exhibit oestrogen receptor (ER) expression [11,14], similar to sporadic tumours.

Fisher and colleagues have recently reported an approximate 50% reduction of breast cancer incidence with 5 years of tamoxifen in a cohort of women at increased risk based on Gail model estimates [6]. The reduction of breast cancer incidence was observed in ER-positive but not ER-negative tumours [6]. Powles did not observe a reduction in breast cancer incidence with tamoxifen use, but the Royal Marsden Tamoxifen Prevention Trial had a high proportion of young women with strong family histories of breast cancer [15]. Given that women with a *BRCA1* mutation may have a higher incidence of ER-negative tumours, it has been postulated that perhaps one of the reasons that the

* Corresponding author. Tel.: +1-913-588-7791; fax: +1-913-588-3679.

E-mail address: cfabian@kumc.edu (C.J. Fabian).

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Royal Marsden Tamoxifen Prevention Trial failed to find a reduction in breast cancer incidence with tamoxifen use was that it may have included a substantial proportion of *BRCA1* mutation carriers [6,15,16]. If *BRCA1* mutation carriers exhibit a predominately ER-negative precancerous phenotype, a reduction in cancer incidence with preventive tamoxifen use would not be anticipated [17]. Molecular and morphological characterisation of precancerous tissue from mutation carriers has not been previously reported and is of interest, both in assisting these women in choosing between current prevention options, as well as in developing new prevention strategies.

Our group at the University of Kansas Medical Center, has previously reported that non-genetically characterised women at increased risk for breast cancer on the basis of family history, a prior precancerous biopsy or prior breast cancer (this will be used to define high risk women throughout this paper) have an increased prevalence of random peri-areolar fine needle aspiration (FNA) evidence of hyperplasia with and without atypia, as well as expression of several other biomarkers (ER, epidermal growth factor receptor (EGFR), p53, Her-2/neu and DNA aneuploidy) [18,19] compared with low-risk controls. In addition, it has been found that these high-risk women with random FNA evidence of hyperplasia with atypia have a higher incidence of short-term cancer development than those high-risk women without atypia [20]. Expression of one or more of the biomarkers ER, p53 or EGFR is also significantly associated with hyperplasia with atypia [20].

Consequently, phase II chemoprevention trials utilising high-risk women with random FNA evidence of hyperplasia with and without atypia have been initiated using FNA cytology as the main surrogate endpoint biomarker [21].

It is currently unknown what proportion of high-risk women entering chemoprevention trials will harbour *BRCA1*/*BRCA2* mutations, whether mutation carriers will exhibit the same morphological and molecular biomarker distribution as non-mutation carriers, or whether these biomarkers can be modulated as readily as those from non-mutation carriers. The University of Kansas Medical Center has recently completed a phase II chemoprevention trial of 6 months of difluoromethylornithine (DFMO) versus placebo in a high-risk cohort who were not genetically characterised at study entry. Subjects were genetically characterised at the completion of their study participation. In this manuscript, we present the prevalence of *BRCA1*/*BRCA2* germ line alterations in women participating in the trial and correlate their random peri-areolar FNA cytology and biomarker patterns at entry with mutation status. As the study remains blinded, the effect (if any) of mutation status on modulation of biomarker patterns cannot be determined at this time.

2. Patients and methods

2.1. Eligibility for chemoprevention trial

2.1.1. Cohort selected to undergo peri-areolar fine needle aspiration (FNA)

Preliminary eligibility for the DFMO chemoprevention trial was based on random periareolar FNA evidence of hyperplasia with atypia or hyperplasia without atypia but with expression of p53 or EGFR. Women were considered candidates for FNA if they had (1) one or more first degree or multiple second degree relatives with breast cancer; (2) prior biopsy evidence of hyperplasia with atypia or lobular carcinoma *in situ* (LCIS); (3) multiple prior biopsies showing proliferative breast disease; or (4) prior treated ductal carcinoma *in situ* (DCIS) or invasive cancer. Women with prior DCIS or invasive cancer had only the contralateral breast aspirated and must have completed all treatment at least 1 year prior to entry.

Women selected for aspiration were generally between the ages of 30 and 55 years. Women younger than 30 years could be aspirated if they were within 10 years of the age of onset of breast cancer in a close relative and those older than 55 years could be entered if they had dense mammograms or recent biopsy evidence of proliferative breast disease. Both pre- and postmenopausal women were eligible.

2.1.2. Concomitant hormonal therapy

Hormone replacement or oral contraceptive therapy was allowed provided a woman had continuous use for $\geqslant 6$ months at the time of initial aspiration and provided she agreed to continue use throughout the trial period.

2.1.3. Mammogram and clinical breast exam

An ACR class I–III mammogram was required within 6 months prior to entry. A woman with a class III mammogram could be entered provided a biopsy was not felt to be necessary, or a biopsy was performed and cancer was not detected in the aspirated breast. In addition, a clinical breast examination must be normal or thought not to warrant biopsy prior to trial entry.

2.1.4. Assessment of cytology and biomarkers

Methodology for random periareolar FNA has been previously published by our group [22]. FNA was performed during days 1–14 of the menstrual cycle for premenopausal women or at any time for peri- and post-menopausal women. Eight to ten aspirates were obtained from each breast using a 21-gauge $1\frac{1}{2}$-inch needle and 10-cc syringe. Cells were pooled in 5 cc of ice cold RMPI before aliquoting for assays. Cytology slides were prepared using a filter prep. technique and classified as non-proliferative, hyperplasia or hyperplasia

Table 1
Results and associated breast cancer risk based on *BRCA1/2* sequencing[a]

BRCA1/2 results	Definition	Effect on breast cancer risk
Positive: deleterious mutation.	Interferes with protein function.	Lifetime risk increased to 56–87%.
Genetic variant, favour polymorphism.	Presumed, but not proven to be clinically insignificant.	Presumed not to increase risk.
Genetic variant, uncertain significance.	Missense mutation or a mutation in the non-coding sequence whose effect on protein function is not yet known.	Variant's effect on risk has not yet been determined.
No variant detected (negative).	No gene sequence abnormality identified.	No change of risk.

[a] Reported by Myriad Genetic Laboratories, Inc.

with atypia [19]. Only subjects with hyperplasia with or without atypia were eligible for the phase II chemoprevention study.

Two-thirds of the cells obtained at aspiration were aliquoted for molecular assays including EGFR, p53, and proliferating cell nuclear antigen (PCNA). In addition, ER and Her-2/neu were performed if there was sufficient material available. Immunochemistry slides were prepared by cytospin techniques and scored for expression using previously reported methodology [22,23].

2.1.5. Gail risk assessment

Assessment of risk for development of DCIS or invasive breast cancer at 10 years was performed for each entrant using the modified model as proposed by Gail [24]. This risk estimate was not used to determine eligibility.

2.1.6. Genetic counselling and testing

Genetic counselling and testing was not required to enter the trial. Counselling was generally performed in the fourth month of study. Blood was drawn at study exit for genetic testing. Women were offered free and confidential genetic testing and were consented if willing to undergo *BRCA1/2* gene sequencing. Full gene sequencing of *BRCA1* and *BRCA2* was performed by Myriad Genetic Laboratories, Inc. (BRCAnalysis™). This methodology has an analytical sensitivity and specificity of $\geqslant 99\%$, but is limited by the inability to detect large chromosomal rearrangements and deletions. Furthermore, since sequencing picks up all genetic alterations in the coding region, polymorphisms and uncertain variants were detected in addition to known deleterious mutations (Table 1).

Results of the testing were given to the patient unless the patient declined to receive them. Follow-up counselling was performed at the time the results were given. Additional follow-up was scheduled as needed.

2.1.7. Statistical analysis

Study data were collected in an Access database and statistical analysis was performed using SPSS for Windows (release 9.0, SPSS, Inc., Chicago, IL, USA). Descriptive analyses and contingency table analyses were run on the initial on-study dataset. In addition, contingency table analyses were run on the initial data on the basis of genetic status.

3. Results

3.1. Family history and genetic status

119 women entered the phase II chemoprevention trial between June 1997 and May 1999. Of the 119 women entering the DFMO chemoprevention trial, 75% had at least 1 first- or second-degree relative with breast cancer, 20% had a prior precancerous biopsy, 9% had prior breast cancer and 13% fit into more than one of these categories. None of the subjects or subject's families had previously been genetically characterised. 116 (97%) of the study cohort agreed to undergo testing. 26 of the 116 women (22%) tested had a genetic alteration in *BRCA1* and/or *BRCA2*. Table 2 describes the alterations identified. Table 3 describes the 28 specific alterations found in this study population (some were detected in more than one individual).

Each of the 3 women with deleterious mutations had a family history which met the American Society of Clinical Oncology (ASCO) criteria for genetic testing. ASCO criteria includes the following: (1) multiple cases of early onset breast cancer; (2) ovarian cancer with a family history of breast or ovarian cancer; (3) breast and ovarian cancer in the same woman; (4) bilateral

Table 2
28 *BRCA1/2* germ line alterations in 26 subjects[a]

	Deleterious mutation	Variants of uncertain significance	Favour polymorphism
BRCA1	3	6	2
BRCA2	0	13	4

[a] 3 subjects had more than one germ line alteration detected.

Table 3
Specific *BRCA1/2* variants detected

Gene	Result	Variant
BRCA1	Deleterious	3875del4
BRCA1	Deleterious	M1775R
BRCA1	Deleterious	E908X
BRCA1	Uncertain significance	R1028C
BRCA1	Uncertain significance	P1099L
BRCA1	Uncertain significance	R1699Q
BRCA1	Uncertain significance	H816R
BRCA1	Uncertain significance	P1238L
BRCA1	Uncertain significance	T826K
BRCA2	Uncertain significance	N56T
BRCA2	Uncertain significance	D2438G
BRCA2	Uncertain significance	I2315L
BRCA2	Uncertain significance	R2502C
BRCA2	Uncertain significance	H1561N, IVS16 + 6C > G, V2138F
BRCA2	Uncertain significance	L2106P
BRCA2	Uncertain significance	IVS16 + 7T > A
BRCA2	Uncertain significance	A2717S
BRCA2	Uncertain significance	K1180R
BRCA2	Uncertain significance	D935N
BRCA2	Uncertain significance	T598A
BRCA2	Uncertain significance	E462G
BRCA2	Uncertain significance	R2502H
BRCA1	Favour polymorphism	R1347G
BRCA1	Favour polymorphism	R1347G
BRCA2	Favour polymorphism	K3326X
BRCA2	Favour polymorphism	D1420Y
BRCA2	Favour polymorphism	D1420Y
BRCA2	Favour polymorphism	I3412V

breast cancer; (5) Ashkenazi Jewish heritage; and (6) male breast cancer [25]. One subject had 2 first-degree and 4 second-degree relatives with breast and ovarian cancer; 1 subject had 3 first-degree and 3 second-degree relatives with breast cancer; and 1 subject had 2 first-degree and 5 second-degree relatives with breast cancer. For those women with variants of uncertain significance, 60% had a family history that met current ASCO criteria for clinical genetic testing.

3.2. Demographic features

Median age of the study cohort was 47 and the median 10-year Gail predicted probability of breast cancer development was 3.9%. 59% were premenopausal at entry and 4% were minority. No differences were observed between women with and without germ line alterations in *BRCA1/2* (Table 4).

3.3. Cytology and biomarkers

Baseline cytology and biomarker expression is listed in Table 5. There were no significant differences between those with and without a germ line alteration. Although a higher proportion of subjects with a *BRCA1/2* alteration had baseline hyperplasia with atypia, (58 versus 48%) this difference was not statistically significant. The proportion of subjects with a *BRCA1/2* alteration who exhibited ER expression in their FNA was 46% compared with 44% of subjects without a *BRCA1/2* alteration and p53 expression was 58 versus 42%, neither of which were statistically significant.

3.4. Subsequent cancer detected

Breast cancer was subsequently detected in 5 women who participated in the DFMO trial, in addition 1 subject was diagnosed with ovarian cancer (Table 6). 3 of the 5 women with breast cancer had at entry random FNA evidence of hyperplasia with atypia and 2 had

Table 4
Demographic features versus genetic status

	Median age (years)	Median 10-year Gail risk	Premenopausal (%)	Minority (%)
Cohort $n = 116$[a]	47	3.9	59	4
No germ line variant $n = 90$	46	3.9	58	3.5
BRCA1/2 alteration $n = 26$	48	4.0	62	8

[a] 3 women declined genetic testing.

Table 5
Cytology and biomarker expression versus genetic status

	Hyperplasia with atypia (%)	ER (≥1+) (%)	EGFR (≥2+) (%)	p53 (≥2+) (%)	Her-2/neu (≥2+) (%)
Cohort $n = 116$[a]	51	42	71	49	55
No germ line variant $n = 90$	48	44	75	42	55
BRCA1/2 alteration $n = 26$	58	46	69	58	54
BRCA1 alteration $n = 10$	60	40	80	50	70
BRCA2 alteration $n = 16$	56	44	63	56	44

[a] 3 women declined testing.

Table 6
Cancer development versus genetic status

Cancer type	Family history (yes or no)	On-study FNA cytology	Time since on-study FNA (months)	Germ line alteration
Breast	Yes	Hyperplasia w/atypia	6	*BRCA2*-favour polymorphism
Breast	No	Hyperplasia w/atypia	15	None
Breast	No	Hyperplasia	25	None
Breast	Yes	Hyperplasia w/atypia	17	None
Breast	Yes	Hyperplasia	22	*BRCA2* uncertain
Ovarian	No	Hyperplasia w/atypia	21	None

BRCA2 variants detected (one of unknown significance and one presumed to be a polymorphism).

4. Discussion

We have demonstrated that the vast majority of women entering a phase II chemoprevention trial will agree to undergo genetic testing when counselling and testing are free and anonymous and the results are kept confidential. 97% of trial participants underwent testing; and of those who were tested, 22% were identified as having a genetic alteration, although only 3% were identified as carrying a known deleterious mutation. 16% of the subjects were identified with uncertain variants and 5% had variants presumed to be polymorphisms.

Cytology and biomarker expression was not different for subjects identified with germ line alterations versus those without germ line alterations. Since all subjects undergoing genetic testing were first required to have hyperplasia with or without atypia as a condition for prevention trial entry, no conclusions can be drawn as to what type of random FNA cytology and biomarker patterns a group of unselected women with germ line alterations in *BRCA1* or *BRCA2* might exhibit. Too few women with definite deleterious mutations were entered into the DFMO phase II chemoprevention trial to be able to address the question of whether mutation carriers are more or less likely than non-mutation carriers to undergo favourable morphological and biomarker modulation with chemoprevention agents. Given the demonstrated feasibility of genetic testing presented here, a prevention trial in which women are stratified by genetic status prior to randomisation could be suggested although such a trial could be logistically difficult given the small number of known deleterious mutation carriers and their widespread geographic distribution. Because confidentially concerns would preclude conducting a trial of only mutation carriers, the trial should also include non-mutation carriers as study participants.

A potential concern is whether genetic testing performed as part of a chemoprevention trial would lead to unnecessary surgery or adverse psychological sequelae particularly if large numbers of mutation carriers of uncertain significance were identified. Although analysis of the quality of life surveys has not yet been completed, we have not found this to be the case judging from responses to an open-ended questionnaire mailed to participants following genetic testing and counselling. Only 1 participant out of the 116 women genetically tested as part of this phase II chemoprevention trial felt she had been adversely affected.

5. Conclusion

Germ line alterations in *BRCA1* and *BRCA2* were found in 22% of subjects who participate in a phase II chemoprevention trial and who agreed to undergo genetic testing. However, only 3/116 (3%) of the study cohort harboured deleterious mutations. There were no apparent differences in FNA cytology and biomarker expression in women with or without a germ line alteration. Due to the inherent limitations of this post-entry genetic testing comparison study, conclusions should be restricted to the cohort participating in the phase II chemoprevention trial and not extended to all high-risk women or to the general population.

References

1. Peto J, Collins N, Barfoot R, *et al*. Prevalence of *BRCA1* and gene mutations in patients with early-onset breast cancer. *J Natl Cancer Inst* 1999, **91**, 943–949.
2. Easton DF, Ford D, Bishop DT, and the Breast Cancer Linkage Consortium. Breast and ovarian cancer incidence in *BRCA-1* mutation carriers. *Am J Hum Genet* 1995, **56**, 265–271.
3. 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**, 676–689.
4. Hartmann LC, Schaid DJ, Woods JE, *et al*. Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. *N Engl J Med* 1999, **340**, 77–84.
5. Burke W, Daly M, Garber J, *et al*. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. *JAMA* 1997, **277**, 997–1003.
6. Fisher B, Costantino JP, Wickerham DL, *et al*. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst* 1998, **90**, 1371–1388.

7. Rebbeck TR, Levin AM, Eisen A, *et al*. Breast cancer risk after bilateral prophylactic oophorectomy in *BRCA1* mutation carriers. *J Natl Cancer Inst* 1999, **91**, 1475–1479.
8. Gail MH, Costantino JP, Bryant J, *et al*. Weighing the risks and benefits of tamoxifen treatment for preventing breast cancer. *J Natl Cancer Inst* 1999, **91**, 1829–1846.
9. Johannsson O, Ranstam J, Borg A, Olsson H. *BRCA1* mutations and survival in women with ovarian cancer. *N Engl J Med* 1997, **336**, 1255–1256.
10. Breast Cancer Linkage Consortium. Pathology of familial breast cancer: differences between breast cancers in carriers of *BRCA1* or *BRCA2* mutations and sporadic cases. *Lancet* 1997, **349**, 1505–1510.
11. Loman N, Johannsson O, Bendahl PO, Borg A, Ferno M, Olsson H. Steroid receptors in hereditary breast carcinomas associated with *BRCA1* or *BRCA2* mutations or unknown susceptibility genes. *Cancer* 1998, **83**, 310–319.
12. Phillips KA, Andrulis IL, Goodwin PJ. Breast carcinoma arising in carriers in *BRCA1* or *BRCA2*: are they prognostically different? *J Clin Oncol* 1999, **17**, 3653–3663.
13. Marcus JN, Watson P, Page DL, *et al*. Hereditary breast cancer: pathobiology, prognosis, and *BRCA1* and *BRCA2* gene linkage. *Cancer* 1996, **77**, 697–709.
14. Verhoog LC, Brekelmans CTM, Seynaeve C, *et al*. Survival in hereditary breast cancer associated with germline mutations of *BRCA2*. *J Clin Oncol* 1999, **17**, 3396–3402.
15. Powles T, Eeles R, Ashley S, *et al*. Interim analysis of the incidence of breast cancer in the Royal Marsden Hospital tamoxifen randomised chemoprevention trial. *Lancet* 1998, **352**, 98–101.
16. Osborne MP. Chemoprevention of breast cancer. *Surg Clin North Am* 1999, **79**, 1207–1221.
17. Alberg AJ, Visvanathan K, Helzlsouer KJ. Epidemiology, prevention, and early detection of breast cancer. *Curr Opin Oncol* 1998, **10**, 492–497.
18. Fabian CJ, Zalles C, Kamel S, *et al*. Prevalence of aneuploidy, overexpressed ER and overexpressed EGFR in random breast aspirates of women at high and low risk for breast cancer. *Breast Cancer Res Treat* 1994, **30**, 263–274.
19. Zalles C, Kimler BF, Kamel S, McKittrick R, Fabian CJ. Cytologic patterns in random aspirates from women at high and low risk for breast cancer. *The Breast J* 1995, **1**, 343–349.
20. Fabian CJ, Kimler BF, Zalles CM, Kamel K. Biomarkers for prediction of breast cancer and for use in breast cancer chemoprevention trials. *9th International Congress on Anti-Cancer Treatment*, Paris, France, 2–5 Feb 1999.
21. Fabian CJ, Kimler BF, Elledge RM, Grizzle WE, Beenken SW, Ward JH. Models for early chemoprevention trials in breast cancer. *Hematol/Oncol Clinics of North America* 1998, **12**, 993–1017.
22. Fabian CJ, Kamel S, Kimler BF, McKittrick R. Potential use of biomarkers in breast cancer risk assessment and chemoprevention trials. *The Breast J* 1995, **1**, 236–242.
23. Fabian CJ, Zalles C, Kamel S, Zeiger S, Simon C, Kimler BF. Breast cytology and biomarkers obtained by random fine needle aspiration: use in risk assessment and early chemoprevention trials. *J Cell Biochem* 1997, **28**(Suppl.), 101–110.
24. Gail MH, Brinton LA, Byar DP, *et al*. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *J Natl Cancer Inst* 1989, **81**, 1879–1886.
25. ASCO Curriculum: Cancer Genetics & Cancer Predisposition Testing: 1998.