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BRCA2 Mutations in 154 Finnish Male Breast Cancer Patients[1]

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Abstract

The etiology and pathogenesis of male breast cancer (MBC) are poorly known. This is due to the fact that the disease is rare, and large-scale genetic epidemiologic studies have been difficult to carry out. Here, we studied the frequency of eight recurrent Finnish *BRCA2* founder mutations in a large cohort of 154 MBC patients (65% diagnosed in Finland from 1967 to 1996). Founder mutations were detected in 10 patients (6.5%), eight of whom carried the *9346(−2) A>G* mutation. Two novel mutations (*4075 delGT* and *5808 del5*) were discovered in a screening of the entire *BRCA2* coding region in 34 samples. However, these mutations were not found in the rest of the 120 patients studied. Patients with positive family history of breast and/or ovarian cancer were often *BRCA2* mutation carriers (44%), whereas those with no family history showed a low frequency of involvement (3.6%; $P < .0001$). Finally, we found only one Finnish MBC patient with *999 del5*, the most common founder mutation in Finnish female breast cancer (FBC) patients, and one that explains most of the hereditary FBC and MBC cases in Iceland. The variation in *BRCA2* mutation spectrum between Finnish MBC patients and FBC patients in Finland and breast cancer patients in Iceland suggests that modifying genetic and environmental factors may significantly influence the penetrance of MBC and FBC in individuals carrying germline *BRCA2* mutations in some populations.

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Keywords: *BRCA2*, mutation, male breast cancer, population, penetrance.

Introduction

In men, breast cancer is approximately 100 times less common than in women. Breast cancer accounts for approximately 1% of all cancers in men. The incidence rates of male breast cancer (MBC) are lower than one case per 100,000 man-years in most populations [1]. In Finland, the age-adjusted incidence of MBC was 0.4 per 100,000 person-years in 2001, with about 13 cases diagnosed annually [2].

Genetic risk factors of MBC include Klinefelter's syndrome [3,4], as well as mutations of the androgen receptor [5,6], *BRCA1* [7,8], and *CHEK2* genes [9]. The strongest known risk factors for MBC are germline mutations in the *BRCA2* gene [7,10–22]. The reported percentage of *BRCA2* mutations in MBC patients has varied significantly (4–40%) among the published studies [13,21] (Table 1). Some of this variation may be due to the small numbers of the study subjects, different patient ascertainment criteria, as well as the variability in mutation detection methodology.

In Finland, 15 *BRCA1* and 8 *BRCA2* mutations have been reported in FBC patients. Fourteen of these, seven in both genes, represent recurrent founder mutations that account for the majority of all detected mutations [23–30]. However, the frequency of *BRCA2* germline mutations in Finnish MBC patients has remained unknown.

Here, we determined the frequency of eight previously described Finnish *BRCA2* mutations in 154 MBC patients collected in Finland over a 30-year period using both blood and paraffin-embedded samples. This represents the largest study so far reported on MBC and *BRCA2* mutations. In addition, in a subset of 34 patients for whom a blood specimen was available, we analyzed the entire coding region of the *BRCA2* gene using protein truncation test (PTT) and denaturing high-performance liquid chromatography (DHPLC).

Materials and Methods

Sample Collection

We reviewed all men with an ICD code for breast cancer notified to the nationwide, population-based Finnish Cancer

Abbreviations: DHPLC, denaturing high-performance liquid chromatography; FBC, female breast cancer; MBC, male breast cancer; PTT, protein truncation test

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Table 1. Frequency of *BRCA2* Mutations in MBC Patients According to Family History of Breast–Ovarian Cancer.

Study Population	Number of Patients	Screening Strategy	Percentage of *BRCA2* Mutation Carriers*		Reference
			All Patients	Patients with Positive Family History	
Canada	14	Entire gene	14 (2/14)	29 (2/7)	[10]
France	12†	Entire gene	25 (3/12)	25 (3/12)	[11]
Hungary	18	Entire gene	33 (6/18)	0 (0/4)	[12]
Iceland	30	Founder	40 (12/30)	90 (9/10)	[13]
Israel	124	Founder	12 (15/124)	–	[7]
Italy	25	Entire gene	12 (3/25)	29 (2/7)	[14]
Poland	37	Entire gene	11 (4/37)	20 (1/5)	[15]
Spain	17‡	Entire gene	18 (3/17)	33 (3/9)	[16]
Sweden	34	Entire gene	21 (7/34)	20 (1/5)	[17]
UK	28	Entire gene	7 (2/28)	10 (1/10)	[18]
UK	33§	Entire gene	36 (12/33)	40 (12/30)	[19]
UK	94	Entire gene	5 (5/94)	16 (3/19)	[20]
USA	54	Entire gene	4 (2/54)	6 (1/16)	[21]
USA	50	Entire gene	14 (7/50)	15 (6/40)	[22]
Total	570		7 (83/570)		
Finland	154	Founder/entire gene	8 (12/154)	44 (7/16)	Present study

*Missense mutations with unknown significance excluded.
† Only cases with positive family history were included.
‡ Six females with breast cancer and a first-degree relative with MBC included.
§ Seventeen females with breast cancer and a first-degree or second-degree relative with MBC included.

Registry between 1967 and 1996, and those 237 with microscopically confirmed diagnosis of carcinoma were eligible for the study. We linked the personal identification codes of these MBC patients with data from the Finnish Population Register Centre, providing us with additional information on vital status, possible date of death, or emigration. All first-degree relatives of the MBC patients, and second-degree relatives when possible, were then identified from the population and parish registries. Cancer diagnoses of the relatives were also identified by linking the personal identification codes of the relatives back to the Cancer Registry. The study protocol was approved by the Ethical Committee of the Tampere University Hospital and the Ministry of Social Affairs and Health in Finland.

Of 237 patients, 79 (33%) were alive, and they were approached through the attending physicians. We obtained a written informed consent to participate in the study and a blood sample from 37 patients. A questionnaire on malignancies in the family was also received. Paraffin-embedded tissue samples were obtained from 130 patients; most of these patients had died of the disease. In 122 of the paraffin-embedded tumors, breast cancer diagnosis was confirmed by a pathologist (T.K.), leading to abandonment of eight samples.

Mutation Detection

DNA was extracted from blood samples using Puregene (Gentra Systems, Minneapolis, MN) or from paraffin-embedded nonmalignant tissues using QIAamp DNA Mini Kit (Qiagen, Valencia, CA). Sufficient amount of DNA was obtained from 156 samples. Eight *BRCA2* mutations, previously found to be present in the Finnish FBC patients, were analyzed in all 156 samples. The mutations screened were *999 del5*, *4081 insA*, *5797 G>T*, *6495/6496 G>C*, *delCA*, *6503 delTT*, *7708 C>T*, *8555 T>G*, and *9346(−2) A>G* (NCBI RefSeq mRNA accession no. NM 000059). Short (80–150 bp) fragments around the possible mutation sites were amplified by polymerase chain reaction (PCR) using genomic DNA. For each primer set, one primer was 5′-biotinylated.

Mutation detection was done using solid-phase minisequencing (single-base extension reaction) as previously described [31] using streptavidin-coated microtiter plates manufactured from scintillating plastic (Wallac; Perkin Elmer, Turku, Finland) and 1450 MicroBeta PLUS Liquid scintillation counter by the same manufacturer. PCR primers and minisequencing detection primers are available upon request. Mutation-positive and mutation-negative controls as well as a negative control for the PCR reaction were included in each analysis. The results were confirmed by direct sequencing using ABI PRISM 310 Genetic Analyzer and Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer) according to the manufacturer's instructions. Two DNA samples from paraffin-embedded tissues were excluded because of conflicting mutation results.

BRCA2 founder mutation was found in 3 of 37 patients from whom DNA samples from whole blood were available, and thus 34 samples were screened for the presence of additional *BRCA2* mutations. The entire coding region of *BRCA2* was analyzed using PTT for exons 10 and 11, and DHPLC for exons 2 to 9 and 12 to 27, including also the first 300 bp of exons 10 and 11. Samples with truncated PTT bands or aberrant DHPLC chromatograms were sequenced, using a new PCR product as a template [32,33].

Statistical Analyses

Statistical analyses were performed using GraphPad InStat version 2.04a (GraphPad Software, San Diego, CA). Continuous parametric variables were shown as mean and standard deviation, and comparisons were made with Student's *t* test. Associations between categorical

variables were analyzed by Fisher's exact test and chi-square analysis.

Results

Mutations

We screened eight previously described Finnish *BRCA2* founder mutations in 154 MBC patients, and three different mutations [*999 del5*, *7708 C>T*, and *9346(−2) A>G*] were found. Altogether, 10 (6.5%) patients were mutation-positive (Table 2). Eight (5.2%) patients carried the *9346(−2) A>G* mutation.

PTT and DHPLC screening of 34 patients revealed two novel mutations (5.9%): *4075 delGT* and *5808 del5*. Additionally, 12 different silent polymorphisms were detected (data not shown). Subsequently, the *4075 delGT* and *5808 del5* mutations were studied in all 154 samples, but no additional mutation carriers were found. Taken together, *BRCA2* mutations were present in 12 (7.8%) MBC patients.

Characteristics of Mutation Carriers

MBC patients with *BRCA2* mutations were only slightly younger (mean 64.9 years; SD 11.9; range 46–91) than noncarriers (mean 65.7 years; SD 12.4; range 30–94) at the time of diagnosis; this difference did not reach statistical significance. None of the four patients under 40 years, and only two of the 17 patients under 50 years of age at the time of diagnosis, carried a mutation.

Sixteen MBC patients had at least one first-degree or second-degree female relative with breast cancer. Seven of these MBC patients (44%) carried a *BRCA2* mutation, whereas only 5 of 138 (3.6%) patients without family history of breast cancer had a *BRCA2* mutation. This difference was statistically highly significant [$P < .0001$; OR = 20 (95% CI 5–76)].

Three MBC patients had a first-degree or second-degree relative with ovarian cancer, and two of these MBC patients carried *BRCA2* mutations: *4075 delGT* and *9346(−2) A>G* (Table 2). Two mutation-positive MBC patients were found to have relatives with MBC. Additionally, two initially unrelated MBC patients with no *BRCA2* mutations were found to be related with each other (second-degree relatives) in more detailed pedigree analysis. Unfortunately, only tissue samples were available from these patients, and we were not able to screen the entire *BRCA2* gene for mutations.

Discussion

We report here the frequency of eight different, previously found Finnish *BRCA2* mutations in 154 Finnish MBC patients, as well as the screening of the entire coding sequence of the *BRCA2* gene in 34 MBC patients for whom blood samples were available. To our knowledge, this is the largest set of MBC patients included in a systematic screening of *BRCA2* mutations. The patients comprised 65% of all MBC patients identified in Finland over a 30-year period, providing a higher coverage of cases in the source population than in many previous studies.

The overall *BRCA2* mutation frequency was 7.8% in the present study, which is slightly lower than *BRCA2* mutation frequencies in MBC in most previously published studies [7,10–22] (Table 1). However, if we expect the rate of sporadic mutations among the 154 to be the same as among the

Table 2. Characteristics of MBC Patients with *BRCA2* Mutations.

Patient	Mutation	Effect	Age at Diagnosis (years)	Histologic Type (Grade)	Family History of Cancer Among First-Degree and Second-Degree Relatives	
					Type of Relative (Age at Diagnosis)	
					Breast Cancer	Other Cancers
Y170	*999 del5*	Frameshift	46	IDC (3)	S (25), S (45), S (54), S (65, 67)*	M stomach (76), F prostate (63), B lung (64), PA uterine (61), PA fallopian tube (79)
Y047	*4075 delGT*	Frameshift	58	ILC	B (78)	S ovary (67), S cervical (71)
Y099	*5808 del5*	Frameshift	66	IDC (3)	MA (76), PA (83)	MA skin (97), GD leukemia (5), B skin (74), BD chorion (30)
Y021	*7708 C>T*	Nonsense	71	IDC (3)	M (80), S (50)	F skin (NA), S liver (78), SD brain (8)
Y063-17	*9346(−2) A>G*	Splice site	67	IDC (2)	SS (71), SD (36), SD (49), SD (50) a, SD (54) b	SD ovarian (58), D uterine (55), So prostate (60), B colon (87), S laryngeal (NA), SD cervical (39), SD cervical (47) a, SD lymphoma (69) b
Y076	*9346(−2) A>G*	Splice site	64	IDC (3)		
Y084	*9346(−2) A>G*	Splice site	49	IDC (2)	S (73)	F prostate (69), F skin (47), B prostate (58)
Y089	*9346(−2) A>G*	Splice site	66	IDC (2)		
Y095	*9346(−2) A>G*	Splice site	69	IDC (3)	D (44) c, S (80)	D chorion (33) c, B prostate (69)
Y103	*9346(−2) A>G*	Splice site	91	IDC (1)		
Y115	*9346(−2) A>G*	Splice site	57	ILC		
Y157	*9346(−2) A>G*	Splice site	75	IDC (2)		B stomach (84), So prostate (56)

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.
M, mother; F, father; S, sister; B, brother; D, daughter; So, son; GD, granddaughter; PA, paternal aunt; PU, paternal uncle; MA, maternal aunt; SD, sister's daughter; SS, sister's son; BD, brother's daughter; NA, not available.
a, b, c, same individuals.
*Bilateral breast cancer.

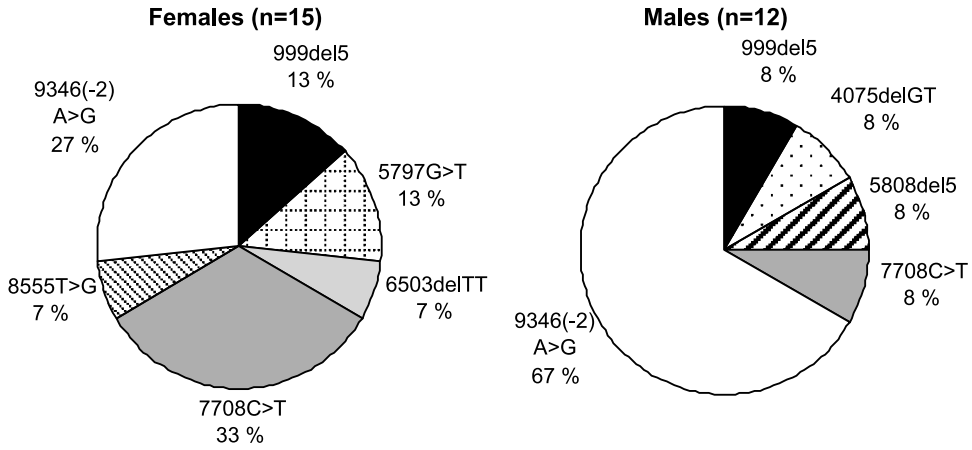

Figure 1. *BRCA2 mutation spectrum among mutation-positive Finnish FBC [27] and MBC populations.*

34 patients screened comprehensively for *BRCA2* mutations, we could estimate that the total mutation burden, including both recurrent and sporadic mutations, is approximately 12% to 13% (6.5% + 5.9%). This is well in line with those found in previous studies, where the entire coding sequence of all patients was analyzed [7,10–22] (Table 1).

In our study, 10.4% (16/154) of the MBC cases had a positive family history of breast cancer, and as many as seven of these 16 patients (44%) carried a *BRCA2* mutation. The percentage of family history–positive MBC patients with *BRCA2* mutations has been found to range from 0% to 90% [7,10–22] (Table 1). In Iceland and Finland where strong founder effects can be found, a larger proportion of family history–positive patients carried a *BRCA2* mutation compared to other populations [13]. In contrast, *BRCA2* mutations were rare (3.6%) among Finnish MBC patients with negative family history of breast and/or ovarian cancer. Many of the other MBC studies have reported similar results, with a range of *BRCA2* positivity in family history–negative cases from 0% to 15% [7,10–22] (Table 1). On the other hand, up to 21% of family-negative MBCs carried a *BRCA2* mutation in Sweden (6/29) and 43% (6/14) in Hungary [17,12].

The frequencies of different *BRCA2* mutations varied greatly among Finnish MBC and female breast cancer (FBC) populations (Figure 1). Among 1035 unselected Finnish FBC patients, 1.8% carried one of the *BRCA1* (0.4%) or *BRCA2* (1.4%) founder mutations [27]. In MBC patients, the most common *BRCA2* mutation was *9346(−2) A>G*, with a frequency of 67% (8/12 *BRCA2* mutation-positive cases). In unselected FBC population, the *9346(−2) A>G* mutation was the second most common mutation with a much lower frequency (27%; 4/15) [28]. The difference in *9346(−2) A>G* mutation frequencies was significant between males and females ($P = .038$; OR = 5.5; 95% CI 1–29). In contrast, only one MBC patient carried the *999 del5* mutation, which is the most common *BRCA1/2* founder mutation in Finland [29]. Up to 40% of all Icelandic MBC patients carries this mutation [13]. All 10 MBC founder mutation carriers originated from the same regions in Finland as the FBC patients carrying the corresponding founder mutations [28]. The low number of *999 del5* mutations cannot be explained by simple selection bias because dozens of samples were available from the two founder areas of this mutation [13]. Our findings and those by Thorlacius et al. [13] therefore point to additional genetic modifier loci, or possibly environmental factors that differentially influence MBC and FBC penetrance among different *BRCA2* mutation carriers.

In summary, we report here a large genetic epidemiologic study that suggested an approximately 13% frequency of *BRCA2* mutations in unselected Finnish MBC patients. Founder mutations account for the majority of these, with one particular mutation [*9346(−2) A>G*] showing a very high frequency among MBC patients. In contrast, the most common Finnish founder mutation in FBC patients (*999 del5*) was seen only in one MBC patient. Interestingly, the same *999 del5* founder mutation accounts for the majority of both male and female hereditary breast cancers in Iceland [13]. Although the numbers of *BRCA2*-positive MBC cases are still relatively small, our results point to some frequency differences of individual *BRCA2* founder mutations when compared to analogous nationwide study of FBC. This may reflect differences in modifier loci, or, equally likely, environmental differences (such as hormonal and dietary) that influence the penetrance of different *BRCA2* mutations between female and male populations as well as between different populations.

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