Selected aspects of genetic counselling for BRCA1 mutation carriers

Jacek Gronwald

International Hereditary Cancer Center, Department of Genetics and Pathology, Szczecin, Poland

Key words: BRCA1, breast cancer, ovarian cancer, risk factors

Corresponding author: Jacek Gronwald, International Hereditary Cancer Center, Department of Genetics and Pathology, Szczecin, Poland, Email: jgron@uoo.univ.szczecin.pl

Submitted: 12 February 2007
Accepted: 14 February 2007

Abstract

This work consists of six parts based on seven manuscripts dealing with some aspects of genetic counselling for BRCA1 mutation carriers. It was demonstrated that the risk of breast and ovarian cancer in first-degree relatives of BRCA1 mutation carriers depends on the type of mutation and is higher in the younger generation. It was also shown that risk of breast cancer, but not of ovarian cancer, is related to cancer type of the proband. These factors should be taken into account when assessing risk of breast and ovarian cancer in relatives of BRCA1 mutation carriers. It was observed that longer breast-feeding, physical activities delaying menarche, preventive oophorectomy, administration of tamoxifen to patients with intact genital tract, and use of contraceptives reduce the risk of breast and ovarian cancer. All these possibilities should be presented to BRCA1 mutation carriers within the framework of cancer risk reduction options. It was also observed that there may be some preference in transmission of the mutant allele to female offspring of BRCA1 founder mutation carriers. Environmental factors appear also to interfere with transmission. The male to female ratio in offspring of BRCA1 mutation carriers is the same as for the general population. As for the consequences of simplified two-stage genetic counselling, the first psychological reaction of a female to the fact that she is a carrier of the BRCA1 mutation is negative. However, understanding that the risk of cancer is high persuades the woman to embrace preventive options. 98% of BRCA1 mutation carriers disclosed during population screening initiated and promoted by the media are convinced of the value of genetic testing. Simplified two-stage genetic counselling appears to be a useful approach promoting increased turnout for BRCA1 mutation testing.

Introduction

Genetic counselling is a process which should explain to the patient all problems caused by development of hereditary disease in the family or risk of such disease [1]. Within counselling the patient should obtain full information about: the disease, its course, possibilities of treatment, genetics, risk of disease for particular family members including planned/unborn children, proceeding (which takes into account actual knowledge, convictions, life priorities) which allow to apply optimal prophylactics, treatment and adaptation to actual life situation [1, 2]. Dynamic development of molecular genetics made possible the diagnosis of a large number of diseases which hereditary background was until recently unknown. Hereditary neoplasms including breast and ovarian cancers belong to this group of disorders. The oldest report on familial breast cancer was made in about 100 BC in the medical literature of ancient times.
Rome [3]. The first report on familial aggregation of breast cancer in modern times was published in 1866 by Broca, who described 10 cases of breast cancer in four generations of his wife’s family [4]. However, only in the middle of the 1990s was it proved at a molecular level that a significant number of breast and ovarian cancers have hereditary monogenic aetiology [5, 6] and testing of BRCA1 and BRCA2 mutations became a common diagnostic tool to identify persons with high risk of these cancers. It has been estimated that in BRCA1 or BRCA2 carriers the risk of breast cancer reaches up to 80%, and of ovarian cancer 40% [7]. But, what is more important, early prophylactics allow this risk to be decreased to levels slightly exceeding population risk [8-10]. In Poland, thanks to the detection of founder mutations in the BRCA1 gene [11], which constitute a very high ratio of all detectable BRCA1 and BRCA2 mutations [11-16], as well as setting up a network of hereditary cancer units, diagnosis of persons with high risk of breast/ovarian cancers has become relatively cheap, common and in this way very effective. In this aspect we are one of the best diagnosed societies and up to now in the Centre of Szczecin alone about 3500 BRCA1 and BRCA2 carriers have been diagnosed. Detection of carriers creates the need for full genetic counselling of the highest standard.

In this work the results of studies on selected aspects of genetic counselling in BRCA1 carriers have been described.

**Objectives**

1. Assessment of the effect of mutation and tumour location in probands on the risk of breast and ovarian cancer in relatives of BRCA1 mutation carriers.
2. Assessment of the effect of some extra-genetic factors, including age at menarche, parity, duration of breast-feeding, preventive oophorectomy, oral contraceptive use, cigarette smoking and coffee consumption on the risk of breast/ovarian cancer in BRCA1 mutation carriers from Poland.
3. Assessment of the effect of tamoxifen on the risk of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers treated for cancer.
4. Assessment of the probability of transmission of the mutant BRCA1 allele to offspring of the mutation carrier.
5. Assessment of the male to female ratio in offspring of BRCA1 mutation carriers.
6. Assessment of psychological and medical consequences of simplified two-stage genetic counselling in females appearing for BRCA1 gene mutation testing.

**Material, methods and results**

**Assessment of the effect of mutation and tumour location in probands on the risk of breast and ovarian cancer in relatives of BRCA1 mutation carriers**

(based on publication: Gronwald J et al. Cancer risk in first degree relatives of BRCA1 mutation carriers: effects of mutation and probands disease status. J Med Genet 2006; 43: 424-428)

Breast and ovarian cancer risk in BRCA1 mutation carriers reach up to 80% and 40%, respectively [7]. However, it is not precisely known which factors influence BRCA1 penetrance. Therefore estimation of cancer risk for a patient with a particular mutation is difficult. Possibly, with mutation additional modifying factors are inherited which influence cancer risk. In this scenario, it should be expected that risk is influenced if the proband was affected with breast or ovarian cancer. There is also a lack of precise data on how mutation type influences BRCA1 gene penetrance.

**Materials and methods**

In the course of a national breast cancer survey we identified 4596 women diagnosed with breast cancer at the age of 50 or below at one of 18 centres in Poland from 1996 to 2003. We were able to obtain a DNA sample for BRCA1 analysis from 3568 of these women. A total of 609 patients with ovarian cancer were interviewed from 1999 to 2004 at eight centres in Poland. The three Polish founder BRCA1 mutations (5382insC, c61G, 4153delA) were identified in 273 cases. A mutation was present in 198 patients with breast cancer and 75 patients with ovarian cancer.

We estimated the age-specific breast, ovarian and total cancer risks for first-degree relatives of mutation carriers for each mutation separately, using Kaplan-Meier survival analyses. Patients were considered to be at risk of cancer from birth until either the development of cancer, death from another cause or the date of patient interview. Penetrance curves were compared for mothers and sisters. The study was approved by the ethics board of the Pomeranian Medical University.

**Results**

The risk of breast cancer for all female first-degree relatives of all mutation carriers was estimated to be 33%, and the ovarian cancer risk 15%. The cumulative risks of cancer among first-degree relatives of the BRCA1 mutation carriers are shown in Table 1.

We observed moderate differences in cancer risk for the subgroups of relatives with each of the three different mutations (Tables 1 and 2).
Selected aspects of genetic counselling for BRCA1 mutation carriers

### Table 1. Estimated cumulative risk of breast, ovarian and other cancer in first-degree relatives of BRCA1 founder mutation carriers

| Parameters | Cancer site in relative | BRCA1 mutation type | First-degree relatives with cancer/total | Cancer risk to age of 50 | Cancer risk to age of 75 |
|------------|-------------------------|---------------------|------------------------------------------|-------------------------|-------------------------|
|            | breast                  |                     |                                          |                         |                         |
|            |                         | 5382insC            | 60/557                                   | 0.15                    | 0.29                    |
|            |                         | C61G                | 32/226                                   | 0.23                    | 0.46                    |
|            |                         | 4153delA            | 8/68                                     | 0.25                    | 0.25                    |
|            |                         | total               | 100/851                                  | 0.18                    | 0.33                    |
|            | ovary                   |                     |                                          |                         |                         |
|            |                         | 5382insC            | 29/557                                   | 0.04                    | 0.17                    |
|            |                         | C61G                | 3/226                                    | 0.01                    | 0.05                    |
|            |                         | 4153delA            | 6/68                                     | 0.08                    | 0.38                    |
|            |                         | total               | 38/851                                   | 0.03                    | 0.15                    |
|            | females                 |                     |                                          |                         |                         |
|            |                         | 5382insC            | 22/557                                   | 0.03                    | 0.15                    |
|            |                         | C61G                | 17/226                                   | 0.05                    | 0.36                    |
|            |                         | 4153delA            | 1/68                                     | 0.02                    | 0.02                    |
|            |                         | total               | 40/851                                   | 0.04                    | 0.19                    |
|            | other                   |                     |                                          |                         |                         |
|            |                         | 5382insC            | 111/557                                  | 0.22                    | 0.61                    |
|            |                         | C61G                | 52/226                                   | 0.29                    | 0.87                    |
|            |                         | 4153delA            | 15/68                                    | 0.36                    | 0.66                    |
|            |                         | total               | 178/851                                  | 0.25                    | 0.67                    |
|            | any                     |                     |                                          |                         |                         |
|            |                         | 5382insC            | 47/553                                   | 0.03                    | 0.36                    |
|            |                         | C61G                | 16/193                                   | 0.05                    | 0.33                    |
|            |                         | 4153delA            | 7/70                                     | 0.00                    | 0.39                    |
|            |                         | total               | 70/816                                   | 0.03                    | 0.35                    |

In the Cox proportional hazard model, the breast cancer risk for relatives of women with the missense mutation C61G was about 40% higher than that conferred by the more common mutation 5382insC. Differences in risk with different mutations were also seen for ovarian cancer. Only 5% of the female relatives of the C61G mutation carriers were affected with ovarian cancer, and this was three times lower than the risk relative to the 5382insC mutation. Risk of ovarian cancer in first-degree relatives of 4153delA carriers was 38% – it was almost twice as high as in relatives of 5382insC carriers.

### Table 2. Cancer risk in relatives depending on type of mutation in proband

| Mutation in proband | Breast cancer | Ovarian cancer | Any cancer |
|---------------------|---------------|----------------|------------|
|                     | RR 95% CI p   | RR 95% CI p   | RR 95% CI p |
| 5382insC (n = 557)  | 1.00          | 1.00           | 1.00       |
| 4153delA (n = 68)   | 1.12 0.53-2.34 | 1.85 0.77-4.47 | 1.17 0.68-2.01 |
| C61G (n = 226)     | 1.44 0.93-2.21 | 0.28 0.09-0.93 | 1.28 0.92-1.78 |

RR – relative risk; CI – confidence interval; p – statistical significance; n – number of cases.

In the Cox proportional hazard model, the breast cancer risk for relatives of women with the missense mutation C61G was about 40% higher than that conferred by the more common mutation 5382insC. Differences in risk with different mutations were also seen for ovarian cancer. Only 5% of the female relatives of the C61G mutation carriers were affected with ovarian cancer, and this was three times lower than the risk relative to the 5382insC mutation. Risk of ovarian cancer in first-degree relatives of 4153delA carriers was 38% – it was almost twice as high as in relatives of 5382insC carriers.
We compared the risk of cancer in sisters and mothers of the probands to establish if the risk appears to be changing with time. For both breast and ovarian cancer the lifetime risk for sisters exceeded that of mothers (Table 3).

Risk level was analyzed also with respect to cancer type in the proband. It was observed that breast cancer risk in first-degree relatives of a proband with a BRCA1 mutation is significantly higher if the proband was affected with breast cancer than affected with ovarian cancer, whereas ovarian cancer risk in first-degree relatives was similar if the proband was affected with breast or ovarian cancer (Table 4).

Assessment of the effect of some extra-genetic factors, including age at menarche, parity, duration of breast-feeding, preventive oophorectomy, oral contraceptive use, cigarette smoking and coffee consumption on the risk of breast/ovarian cancer in BRCA1 mutation carriers from Poland

(based on publication of Gronwald J et al. Influence of selected lifestyle factors on breast and ovarian cancer risk in BRCA1 mutation carriers from Poland. Breast Cancer Res Treat 2006; 95: 105-109)

Several environmental and lifestyle factors are believed to contribute to the development of breast cancer in the general population and it is of interest to establish if these factors operate among mutation carriers as well [17-31]. To evaluate the effects of age of menarche, parity, breast-feeding, oophorectomy and oral contraceptive use, as well as smoking and coffee consumption, on the risks of breast and ovarian cancer, we conducted a matched case-control study of Polish women with BRCA1 mutations.

Materials and methods

There were 1482 BRCA1 carriers who completed a baseline questionnaire. There were 591 women affected with breast cancer including 41 who were also affected with ovarian cancer, 189 women affected with ovarian cancer and 734 women unaffected with either cancer. To study the influence of selected factors on the risk of breast cancer, a matched case-control study was done. Each case affected with breast cancer was matched to a healthy control at year of birth (± 1 year). After matching there were 348 breast cancer patients and matched controls. To study the influence of these factors on ovarian cancer risk a second matched case-control study was done. After matching there were 150 ovarian cancer patients and matched controls. Afterwards, patients affected with both breast and ovarian cancer, and the control group were evaluated for the effects of age of menarche, parity, breast-feeding, oophorectomy and oral contraceptive use, as well as smoking and coffee consumption, making proper analyses. McNemar’s test was used to assess the statistical significance of these univariate comparisons. Paired $t$-test was used to compare continuous variables in cases and controls.
Multivariate odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by conditional logistic regression. All p-values were two-tailed and a p-value of 0.05 was considered to be statistically significant. The study was approved by the local ethics committee.

Results

Results of analyses of selected factors on breast cancer risk are presented in Table 5.

Significant differences between groups of cases and controls were observed for age of menarche, parity and breast-feeding. We estimate that each year of delayed menarche is associated with a 10% decrease in breast cancer risk (OR=0.9; p=0.004). BRCA1 carriers who had more children were significantly more likely to develop breast cancer; every additional birth corresponded with a 20% increase in risk (OR=1.2; p=0.02). Breast-feeding for longer than 1 year was found to be protective (OR=0.5; p=0.02). Bilateral oophorectomy also appeared to be protective (OR=0.4); however, the number of cases and controls who underwent this procedure was small and the difference was not statistically significant (p=0.08). We did not observe significant influences on breast cancer risk for the other studied factors, including coffee or smoking.

Results of analyses of selected factors on ovarian cancer risk are presented in Table 6.

A large protective effect was found with ever use of oral contraceptives (OR=0.4; p=0.04). There were few long-term pill users in Poland. Women who used the pill for more than 2 years experienced an 80% reduction in ovarian cancer risk (OR=0.20; p=0.01), compared to women who had never used it.

Assessment of the effect of tamoxifen on the risk of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers treated for cancer

(based in publication: Gronwald J et al. Tamoxifen and contralateral breast cancer in BRCA1 and BRCA2 carriers: an update. Int J Cancer 2006; 118: 2281-2284)

The protective effect of tamoxifen has been shown to reduce the risk of contralateral breast cancer in

Table 5. The effect of some factors on the risk of breast cancer in BRCA1 mutation carriers

| Parameters                  | Cancer patients (n = 348) | Control group (n = 348) | OR   | 95% CI      | p    |
|-----------------------------|---------------------------|-------------------------|------|-------------|------|
| Year of birth               | 1956.3                    | 1956.7                  | 0.58 |             |      |
| Age at diagnosis            | 41.0                      |                         |      |             |      |
| Age at menarche             | 13.5                      | 13.8                    | 0.9a | 0.8-1.0     | 0.004|
| Parity                      | 2.1                       | 2.0                     | 1.2b | 1.0-1.4     | 0.02 |
| Cigarette smoking           | 46%                       | 46%                     | 1.1  | 0.8-1.5     | 0.69 |
| Coffee consumption          | 74%                       | 78%                     | 0.8  | 0.5-1.1     | 0.21 |
| Oophorectomyc               | 2.0%                      | 3.7%                    | 0.4  | 0.1-1.1     | 0.08 |

Breast-feeding:

|               | never | ≤1 year | >1 year |
|---------------|-------|---------|---------|
|               | 20%   | 58%     | 22%     |
|               | 18%   | 55%     | 27%     |
|               | 1.0   | 0.8     | 0.5     |
|               |       | 0.5-1.3 | 0.3-0.9 |
|               |       | 0.37    | 0.02    |

Contraceptives:

|               | never | ever | ever ≤2 years | ever >2 years |
|---------------|-------|------|---------------|---------------|
|               | 84%   | 16%  | 6%            | 2%            |
|               | 82%   | 18%  | 4%            | 10%           |
|               | 1.0   | 0.8  | 0.9           | 0.8           |
|               |       | 0.5-1.2| 0.5-1.5     | 0.5-1.4     |
|               |       | 0.31  | 0.67         | 0.47         |

CI – confidence interval; n – number of cases; OR – odds ratio; p – statistical significance.

a – per additional year; b – per additional birth; c – prior to diagnosis of breast cancer.
carriers of BRCA1 or BRCA2 mutations [10]. Because of the small number of carriers, the level of protection has not been precisely defined. It is also of interest to establish whether or not there is a protective effect of tamoxifen in women who have previously undergone an oophorectomy and to evaluate the protective effect separately for pre- and postmenopausal women.

**Materials and methods**

Information on patients with hereditary breast cancer was submitted to the study centre by investigators at each of 49 contributing centres in 10 countries. The data centre received information on a total of 2972 cases of invasive breast cancer in carriers of pathogenic BRCA1 or BRCA2 mutations. Among the 2972 cases, there were 611 cases of bilateral breast cancer (20.6%) and 2361 cases of unilateral breast cancer (79.4%). Bilateral cases were excluded from the current study if the first cancer was diagnosed prior to January 1, 1970 (i.e. before tamoxifen was in use (n=180)), if the contralateral cancer occurred within 1 year of the diagnosis of the initial breast cancer (n=47) or if the case was diagnosed with ovarian cancer at any time prior to the contralateral breast cancer (n=28). A total of 356 eligible cases of bilateral breast cancer were identified. Women with unilateral breast cancer in the registry database were eligible to serve as controls. Controls were born within 3 years of the birth date of the case, and were diagnosed with breast cancer at an age within 2 years of the age of the first diagnosis of breast cancer of the case. Cases and controls were carriers of mutations in the same gene (BRCA1 or BRCA2). Cases and controls were also matched for oophorectomy (yes/no—1 or more years prior to the age of second cancer diagnosis in the bilateral case). Women were ineligible to serve as controls if they were diagnosed prior to 1970, if they had a contralateral mastectomy or if they had a diagnosis of ovarian cancer prior to, or during, the follow-up period. For each bilateral case, we attempted to identify one or more unilateral control patients. No woman received tamoxifen prior to the diagnosis of the initial breast cancer. All of the exposures in cases and controls are defined for the time period equivalent to the period before the contralateral cancer of the matched case. Our study was restricted to living cases, because it is only possible to perform mutation

---

**Table 6. The effect of some factors on the risk of ovarian cancer in BRCA1 mutation carriers**

| Parameters               | Cancer patients (n = 150) | Control group (n = 150) | OR   | 95% CI          | p    |
|--------------------------|--------------------------|------------------------|------|-----------------|------|
| Year of birth            | 1951.8                   | 1951.7                 |      |                 |      |
| Age at diagnosis         | 47.5                     |                        |      |                 |      |
| Age at menarche          | 13.7                     | 14.0                   | 0.9a | 0.8-1.0         | 0.09 |
| Parity                   | 2.0                      | 2.2                    | 0.9b | 0.7-1.2         | 0.35 |
| Cigarette smoking        | 49%                      | 45%                    | 1.3  | 0.8-2.3         | 0.25 |
| Coffee consumption       | 73%                      | 77%                    | 0.7  | 0.4-1.3         | 0.21 |
| Oophorectomya            | 2.0%                     | 3.7%                   | 0.4  | 0.1-1.1         | 0.08 |
| Breast-feeding:          |                          |                        |      |                 |      |
| never                    | 22%                      | 19%                    |      |                 |      |
| ≤1 year                  | 58%                      | 58%                    | 1.0  | 0.5-1.9         | 0.97 |
| >1 year                  | 20%                      | 23%                    | 1.0  | 0.4-2.6         | 0.97 |
| Contraceptives:          |                          |                        |      |                 |      |
| never                    | 92%                      | 86%                    |      |                 |      |
| ever                     | 8%                       | 14%                    | 0.4  | 0.2-1.0         | 0.04 |
| ever ≤2 years            | 6%                       | 4%                     | 0.8  | 0.2-2.5         | 0.69 |
| ever >2 years            | 2%                       | 10%                    | 0.2  | 0.1-0.7         | 0.01 |

CI – confidence interval; n – number of cases; OR – odds ratio; p – statistical significance.
a – per additional year; b – per additional birth; c – prior to diagnosis of breast cancer.
Selected aspects of genetic counselling for BRCA1 mutation carriers

The frequency of tamoxifen use was compared between the bilateral cases and unilateral matched controls. The odds ratio for contralateral breast cancer associated with tamoxifen use were adjusted for the other covariates, including other treatments received (radiotherapy and chemotherapy), smoking (ever/never), parity and oral contraceptive use (ever/never). To estimate the protective effect of tamoxifen separately for BRCA1 and BRCA2 carriers, and for women with and without oophorectomy, estimates were generated for these subgroups using the matched subsets. All calculations were done using the SAS Statistical Package. The study was approved by the ethics board of the University in Toronto.

### Results

Tamoxifen use was reported by 12.3% of the bilateral cases and by 24.6% of the unilateral controls \((p = 0.001)\). The univariate odds ratio for tamoxifen use and contralateral breast cancer was 0.45. The results of the multivariate analysis were similar. The protective effect of tamoxifen was similar in both BRCA1 and BRCA2 carriers; among the BRCA1 cases and matched controls, the univariate odds ratio was 0.48 and among the BRCA2 carriers the odds ratio was 0.39 (Table 7).

Cases and controls were matched for oophorectomy status. This was done to evaluate the protective effect of tamoxifen separately for women with and without intact ovaries. The results suggest that tamoxifen is protective against contralateral breast cancer in women with intact ovaries \((OR = 0.44)\). A protective effect was not observed among women who had undergone oophorectomy \((OR = 0.87)\) – Table 8.

It is also of interest to establish whether tamoxifen is protective after natural menopause. The observed protective effects of tamoxifen were similar for women who were diagnosed with their first breast cancer before menopause \((OR = 0.54)\) and those initially diagnosed after menopause \((OR = 0.33)\) – Table 9.

There did not appear to be any residual protection offered by tamoxifen beyond 10 years of the first breast cancer diagnosis (Table 10).

### Assessment of the probability of transmission of the mutant BRCA1 allele to offspring of the mutation carrier

(based on publication: A. Gronwald J et al. Non-random transmission of mutant alleles to female offspring of BRCA1 carriers in Poland. J Med Genet 2003; 40: 719-720. B. Gronwald J et al. Transmission of mutant alleles to female offspring of BRCA1 carriers in Poland. J Med Genet 2005; 42: e40)

A. Constitutional mutations in the BRCA1 gene predispose to an autosomal dominant syndrome of breast

### Table 7. Relationship between tamoxifen and risk of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers

| Parameters | Case \((n = 285)\) | Control \((n = 751)\) | Univariate analysis | Multivariate analysis |
|------------|-----------------|-----------------|-------------------|---------------------|
|            | OR  | 95% CI | p    | OR  | 95% CI | p    |
| All carriers: tamoxifen, n |   |       |     |       |       |
| never | 250 | 566 | 1.00 | 1.00 |
| ever | 35  | 185 | 0.45 | 0.29-0.70 | 0.0004 | 0.47 | 0.30-0.74 | 0.001 |
| BRCA1 carriers: tamoxifen, n |   |       |     |       |       |
| never | 204 | 483 | 1.00 | 1.00 |
| ever | 24  | 138 | 0.48 | 0.29-0.79 | 0.004 | 0.50 | 0.03-0.85 | 0.01 |
| BRCA2 carriers: tamoxifen, n |   |       |     |       |       |
| never | 46  | 83  | 1.00 | 1.00 |
| ever | 11  | 48  | 0.39 | 0.16-0.94 | 0.003 | 0.42 | 0.17-1.02 | 0.05 |

### Table 8. The effect of tamoxifen on the risk of contralateral breast cancer by oophorectomy status (multivariate analysis)

| Parameters     | n  | OR     | 95% CI       | p    |
|----------------|----|--------|--------------|------|
| Oophorectomy   | 26 | 0.83   | 0.24-2.89    | 0.7700 |
| No oophorectomy| 259| 0.44   | 0.27-0.65    | 0.0009 |

CI – confidence interval; n – number of cases; OR – odds ratio; p – statistical significance.
It is expected that 50% of the daughters of women who carry a mutation in BRCA1 should be carriers of this mutation based on the principles of Mendelian transmission. At birth, it is expected that 50% of the children of a carrier parent will inherit a mutant allele. If the mortality in carriers is higher in carriers than in noncarriers, then the proportion of carriers among offspring is expected to decline with age. Similarly, among unaffected women, the proportion of carriers is expected to decline with age. Taking into account these theoretical assumptions, cancer risk for relatives of cancer patients as well as penetrance of the BRCA1 gene are calculated [32, 33]. In this work empirical evaluation of this hypothesis was performed.

### Materials and methods

In total, 387 carrier probands (drawn from three sources: (a) 44 carrier probands were found in 490 consecutive cases of breast cancer diagnosed in women under 50 years of age; (b) 46 carrier probands were found in 347 consecutive ovarian cancer cases diagnosed at any age; and (c) 297 carrier probands were found among women with a family history of breast or ovarian cancer who were referred for genetic counselling) were identified, of whom 247 had one or more daughters. To avoid the possibility of selection bias we included only families in which the mothers received their genetic test result before any of the daughters were tested (218 of 247). Of these 218, 91 mothers had one or more daughters who were tested for the mutation and 127 had daughters who were not tested. The 91 carrier mothers had 141 daughters, of whom 126 were tested (range 1-4 daughters per mother). Four of the daughters had been affected by breast cancer and were excluded. The study was approved by the ethics board of the Pomeranian Medical University in Szczecin.

### Results

The mean age of the daughters was 26.5 years (range 7-50 years) (mutation results were not offered to daughters under the age of 18 years). The prevalence of mutations in the daughters by age is given in Table 11.

### Table 9. The effect of tamoxifen on the risk of contralateral breast cancer by menopausal status (univariate analysis)

| Parameters                          | n   | OR  | 95% CI       | p    |
|-------------------------------------|-----|-----|--------------|------|
| Both cancers premenopausal          | 86  | 0.31| 0.11-0.82    | 0.02 |
| A. All carriers:                    |     |     |              |      |
| – Pre- and postmenopausal           | 259 | 0.44| 0.27-0.65    | 0.0009|
| – Both cancers postmenopausal       | 114 | 0.54| 0.27-1.05    | 0.07 |
| – Both cancers postmenopausal       | 37  | 0.33| 0.11-1.01    | 0.05 |
| B. Natural menopause only:          |     |     |              |      |
| – Pre- and postmenopausal           | 22  | 0.13| 0.02-1.10    | 0.06 |
| – Both cancers postmenopausal       | 13  | 0.45| 0.09-2.37    | 0.34 |

CI – confidence interval; n – number of cases; OR – odds ratio; p – statistical significance.

### Table 10. The effect of tamoxifen on the risk of contralateral breast cancer depending on time from first breast cancer (univariate analysis)

| Years since diagnosis of first primary breast cancer | 1-5 years | 5-10 years | >10 years |
|-----------------------------------------------------|-----------|------------|----------|
| Tamoxifen, any use, n                                | n = 168   | n = 68     | n = 49   |
|                                                     | RR  | 95% CI   | p   | RR  | 95% CI   | p   | RR  | 95% CI   | p   |
| Never                                               | 1.00|          |      | 1.00|          |      | 1.00|          |      |
| Ever                                                | 0.46| 0.27-0.79| 0.005| 0.42| 0.16-1.10| 0.08| 0.99| 0.13-7.61| 0.99|

CI – confidence interval; n – number of cases; OR – odds ratio; p – statistical significance.
In total, 75 of 122 unaffected daughters (61.5%) were carriers of the mutation; 61 would have been expected under a transmission ratio of 50% (p=0.011). Surprisingly, there was no evidence of declining prevalence of mutations with increasing age of the daughters.

Results were similar for each of the three groups of probands. Among the tested daughters of the unselected cases of breast cancer 18 mutations were observed (15 expected), among the daughters of the unselected cases of ovarian cancer 12 mutations were observed (nine expected), and among the daughters of the mothers referred to the genetics clinics 45 carriers were observed (37 expected).

Results were similar for the three mutations studied: among daughters of mothers with the 5382insC mutation 46 carriers were observed (36.5 expected); among daughters of mothers with the G61C mutation 16 mutations were observed (12.5 expected); and among daughters of mothers with the 4153delA mutation 13 mutations were observed (12 expected). For comparison purposes, we also tested 63 sons of the carrier mothers; 30 mutations were found (31.5 expected).

Materials and methods

Two years after the publication of Gronwald et al. [34], similar studies were made by de la Hoya et al. [35] in a Spanish and Dutch cohort, which also observed a higher ratio of carrier daughters (58% in those under 30 years of age). However, Evans et al. found no evidence of non-random transmission in an English cohort [36]. Because of the potential importance of these observations for genetic counsellors and for our understanding of BRCA1 genetics, we repeated this study on an unselected series of breast cancer patients. This study is superior in design to our earlier study in that the mutation carriers were drawn from a pool of unselected breast cancer patients and all first-degree relatives were accounted for and offered genetic testing.

### Table 11. Mutation frequency by age among unaffected daughters of BRCA1 mutation carriers

| Age group | Number of carriers | Number of non-carriers | Percentage of carriers (%) | p |
|-----------|--------------------|------------------------|----------------------------|---|
| 0-19      | 12                 | 14                     | 46.0                       | 0.600 |
| 20-29     | 38                 | 18                     | 68.0                       | 0.008 |
| 30-39     | 16                 | 10                     | 61.5                       | 0.240 |
| 40-50     | 9                  | 5                      | 64.0                       | 0.290 |
| Total     | 75                 | 47                     | 61.5                       | 0.011 |

### Table 12. Number by age of mutation positive and negative first-degree female relatives of BRCA1 mutation carriers (both studies together)

| Year of birth | Number of carriers | Number of non-carriers | Transmission ratio (%) | p |
|---------------|--------------------|------------------------|------------------------|---|
| <1950         | 10                 | 11                     | 48                     | 0.800 |
| 1951-1960     | 40                 | 29                     | 58                     | 0.300 |
| 1961-1970     | 36                 | 28                     | 56                     | 0.300 |
| 1971-1980     | 71                 | 40                     | 64                     | 0.003 |
| >1981         | 42                 | 49                     | 46                     | 0.900 |
| Total         | 199                | 157                    | 56                     | 0.030 |

**Results**

The BRCA1 mutation was present in 57 of the 109 daughters (52%) and in 67 of the 125 (54%) sisters. Of the 125 sisters, 41 had breast cancer (22%). Of these, 23 were tested and 22 were found to be positive. The other 146 sisters were unaffected; of these, 102 were tested and 45 (43%) were positive. Assuming the same distribution of carriers and non-carriers in the 62 untested sisters (18 affected and 44 unaffected), we estimate that 103 of the sisters were positive for the family mutation and 84 were negative (p=0.08). The estimated transmission ratios for daughters was 52% and for sisters 55%. Among women in the first study who were born between 1971 and 1980, there were 38 carriers and 18 noncarriers (transmission ratio 68%). In the present study the proportion was 60% (33 of 55). When the data from the two independent studies were merged, the possibility of an effect by calendar year was supported to a modest degree (Table 12). However, there is no consistent trend here; this was a post hoc comparison, and there were no significant differences between the rows.
Assessment of the male to female ratio in offspring of BRCA1 mutation carriers

To the editor: It has been reported that the RING domain of BRCA1 protein interacts with the Xist RNA in mammalian cells, thereby influencing X chromosome inactivation [37]. Lee et al. showed that in mice, defective X-chromosome inactivation changes the sex ratio of offspring [38]. In this context it has been suggested that BRCA1 insufficiency may reduce the ability of Xist RNA to accumulate along the X chromosome and lead to a skewed sex ratio in children [39]. In light of these findings, we were interested in the results of de la Hoya and colleagues, who reported an increased ratio of female to male offspring of BRCA1 mutation carriers, but not of BRCA2 mutation carriers [40]. It is well known that studies such as these may be influenced by possible ascertainment biases [45], in particular if women with daughters are more likely to seek genetic testing than women with only boys or with no children. Recently, several authors observed only slight, statistically insignificant excesses of female offspring of BRCA1 or BRCA2 carriers, suggesting that the observed sex ratio skew against male births might be due to ascertainment bias [41-44]. Studies in which BRCA carriers are ascertained within a consecutive series of breast or ovarian cancer cases, and are unselected for family histories and sex distribution of children, are ideally suited to study this question as they are free from ascertainment bias [35].

Materials and methods

In the course of a national breast cancer survey, through cancer registries of pathology departments we identified 4596 consecutive women with breast cancer diagnosed before age 50 at one of 18 centres situated throughout Poland from 1996-2003. In each centre affected women offered blood for genetic testing. We were able to obtain a DNA sample for BRCA1 analysis on 3568 of these patients. Three founder mutations in the BRCA1 gene (5382insC, C61G, and 4153delA) which cover about 90% of detectable BRCA1/2 mutations in Poland were studied. Among these women 198 mutation carriers were identified (5.54%). The study was approved by the ethics board of the Pomeranian Medical University in Szczecin.

Results

Through pedigree review we identified 189 sons and 172 daughters of these 198 BRCA1 mutation carrier women. The male to female ratio was 1.10. According to the Polish Main Statistical Office the male to female ratio in the general population at birth is 1.06 [46]. Thus the difference is insignificant ($\nu^2=0.08; p=0.77$).

Assessment of psychological and medical consequences of simplified two-stage genetic counselling in females appearing for BRCA1 gene mutation testing

As the number of preventive options for women at high risk for hereditary breast cancer expands, the demand for testing increases. However, many women do not have ready access to testing because of cost, and many others have not been recognized by their physicians to be candidates for testing. There are many effective methods which allow a reduction of cancer risk for women with hereditary predispositions for breast or ovarian cancer [21-25, 29, 47]. It is a challenge to identify all women in the population who carry a BRCA1 mutation and provide them proper management. To achieve this target many organizational and economic difficulties must be overcome. Beyond difficulties related to costs of genetic testing, which substantially vary in specific populations, there are other obstacles facing: (a) mass access to patients who should be offered precise diagnosis by BRCA1 and BRCA2 testing; (b) proper concentration of genetic counselling on a group of patients of the highest cancer risk.

It is possible to increase women’s awareness about hereditary cancer through the popular press. On the other hand oncological geneticists working in the present obligatory system are unable to provide service to the increased number of patients. Currently, in most clinics, women who are to undergo genetic testing receive at least one personal (one-on-one) counselling session (or a series of sessions) where they receive detailed information regarding what to expect in the event of a positive test. Because personalized counselling is expensive and time-consuming, testing is usually restricted to women who have a high chance of carrying a mutation and who have adequate resources at their disposal (through private or public means). It is well known that because of the low number of children in today’s families, inheritance through the paternal line and incomplete penetrance, mutation carriers very frequently come from families with insignificant family history. Such patients according to frequently applied up to now indications would never be qualified for genetic testing. In this work the consequences of an alternative protocol were studied. In this scenario information about hereditary breast/ovarian cancer and genetic testing was delivered to patients with possible increased cancer risk...
by the popular press; wide indications for BRCA1 testing were applied; expanded genetic counselling was limited to patients with identified BRCA1 mutation or with pedigree data indicating increased cancer risk.

Materials and methods

In October 2001 a popular Polish women’s magazine (Twoj Styl or Your Style) published a supplement dealing with breast cancer. This is among the best known women’s magazines in Poland and has a circulation of 400,000. The issue contained an article dealing with issues surrounding hereditary breast cancer, including the state of genetic testing in Poland and the possible risks and benefits of genetic testing. Various ways of reducing cancer risk were described. In collaboration with the Hereditary Cancer Centre at the Pomeranian Medical University, the publishers of Twoj Styl offered an opportunity for 5000 of their readers to participate in genetic testing at no cost. Women qualified if they were 18 years of age or over and if they had a first- or second-degree relative with breast cancer before age 50 or ovarian cancer at any age, or if they themselves had such history of breast or ovarian cancer. Readers who qualified could clip a coupon inviting them for genetic testing and present the coupon at one of 20 familial cancer outpatient clinics situated throughout the country. When the woman arrived at the clinic she presented her coupon and her indications for testing were confirmed by the local staff. If she qualified she signed a consent form and she gave a blood sample for testing. A brief intake form was completed regarding family history and personal history of cancer. Genetic testing was done for the three founder BRCA1 mutations which are common in the Polish population. A total of 5024 tests were completed between November 2001 and February 2002 at the Hereditary Cancer Centre in Szczecin. Out of these, 198 women (3.9%) were found to carry a BRCA1 mutation, and 1760 women (35%) had stronger family predisposition to breast and/or ovarian cancer. In these patients expanded genetic counselling providing detailed information about cancer risk and preventive options was carried out. Other patients obtained expanded genetic counselling if they wished.

Investigation report

A questionnaire was sent to a sample of the study subjects approximately 1 year after they received the test result. The questionnaire dealt with the knowledge of the test results and the satisfaction with the testing process (available upon request). Women were asked whether or not they valued the testing process and whether they were satisfied with their decision to participate. Women were questioned about cancer prevention practices over the past year. Questionnaires were sent to all 198 women with a positive genetic result and to a random sample of 280 women without mutations. The study was approved by the ethics board of the Pomeranian Medical University in Szczecin.

Results

Among the 5024 women who received testing 198 BRCA1 mutations were identified (3.9%). A questionnaire was received from 126 women with a positive genetic test result (72%). Six carrier women had died and two refused. Twenty-six women were lost to follow-up and 15 did not respond. Of the 126 carriers who responded, 63 women had a past history of cancer and 63 had no history of cancer. A random sample of 280 non-carriers was selected. Of these 173 (62%) returned the questionnaire. Twenty-eight of these (16%) had a past history of breast or ovarian cancer and 145 had no history of cancer.

Upon receiving a positive test result the most common immediate reactions among mutation carriers were worry (36.5%), shock (27%) and sadness (22%). Among non-carriers the most common reactions were relief (63.5%) and happiness (29.5%).

On average, carriers estimated their lifetime risk of breast cancer to be 60.5% and of ovarian cancer to be 48%. Non-carriers with a strong family history estimated their lifetime risk for breast cancer to be 29% on average and estimated their lifetime risk of ovarian cancer to be 22%. Non-familial non-carriers estimated their breast cancer risk on average to be 13% and their ovarian cancer risk to be 8.5%. On average, carriers used preventive measures more frequently than non-carriers (Table 13).

Approximately two-thirds of the carriers and just over one-half of the familial non-carriers had complied with the annual recommendations for breast cancer screening. Compliance was much less for ovarian cancer prevention.

Satisfaction rates among the subjects were very high. 98% of the women indicated that they would recommend genetic testing to other women in their position. The proportion of satisfied women was equally high among carriers (98%) and non-carriers (97%).

Summary of the results

1. The risk of breast and ovarian cancer in first-degree relatives of women with BRCA1 mutation depends on mutation type, and is higher in the younger generation. Additionally, the cancer site diagnosed in the proband influences the risk of breast cancer, but not necessarily for ovarian cancer.
2. In BRCA1 mutation carriers from Poland:
   a) delay of menarche, long-term breast-feeding and
      – with high probability – oophorectomy decreases
      the risk of breast cancer;
   b) carriers who had more children were significantly
      more likely to develop breast cancer;
   c) long-term use of oral contraceptives decreases
      the ovarian cancer risk;
3. Risk of contralateral breast cancer was reduced by
   more than 50% in carriers of BRCA1 and BRCA2
   mutations when tamoxifen was given. Protective effects
   of tamoxifen were observed in carriers before
   menopause and those after menopause. There did
   not appear to be any residual protection offered by
   tamoxifen beyond 10 years of the first breast cancer
   diagnosis.
4. Probably there is slight preferential transmission of
   the mutated allele to daughters of BRCA1 carriers.
   In two independent studies, similar differences in
   transmission of the mutated allele, especially among
   children born in the 1970s, were observed
5. Among children of carriers with the BRCA1 mutation,
   the proportion of boys and girls is as in the whole
   population.
6. First psychological reactions after receiving information
   about being a carrier of BRCA1 mutation are negative.
   Identification of high cancer risk increase use of
   prophylactic measures. 98% of BRCA1 carriers
   identified during action initiated by mass media would
   recommend testing for people in a similar life situation.

Conclusions

1. BRCA1 mutation type and tumour location in the
   proband should be considered in the evaluation of
   breast or ovarian cancer risk. Probably, there are
   modifying genes influencing BRCA1 mutation
   penetrance.
2. Long-term breast-feeding, intensive physical exercise
   which delays first menarche, prophylactic adnexectomy
   and use of contraceptives at the appropriate age
   should be presented to BRCA1 mutation carriers as
   options decreasing breast and/or ovarian cancer risk.
3. In the case of patients with breast cancer and without
   oophorectomy, use of tamoxifen should be presented
   to BRCA1 and BRCA2 mutations carriers as a preven-
   tive treatment of contralateral breast cancer.
4. Environmental conditionings should be taken into
   consideration as a reason for changes in transmission
   degree of the mutant allele of BRCA1.
5. An effect of female carrier state of BRCA1 on male
   to female ratio among offspring is unlikely.
6. To increase access to BRCA1 testing simplified two-
   step genetic counselling may be considered.

References

1. Fraser CF. Genetic counseling. Am J Hum Genet 1974; 26:
   636-661.
2. Mazurczak T. Poradnictwo genetyczne – czym jest i jakim byæ
   powinno. Med Sci Rev Genetyka 2004; 11-17.
3. Lynch HT. Genetics and breast cancer. Van Nostrand-Reinhold,
   New York 1981.
4. Broca P Traite de tumeurs. Asselin, Paris 1866.
5. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K,
   Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W et al.
   A strong candidate for the breast and ovarian cancer susceptibility
   gene BRCA1. Science 1994; 266: 66-71.
6. Thompson D, Easton D; Breast Cancer Linkage Consortium.
   Variation in BRCA1 cancer risks by mutation position. Cancer
   Epidemiol Biomarkers Prev 2002; 11: 329-336.
7. Easton DF, Ford D, Bishop DT. Breast and ovarian cancer
   incidence in BRCA1-mutation carriers. Breast Cancer Linkage
   Consortium. Am J Hum Genet 1995; 56: 265-271.
8. Rebbeck TR, Levin AM, Eisen A, Snyder C, Watson P, Cannon-
   Albright L, Issacs C, Olopade O, Garber JE, Godwin AK, Daly
   MB, Narod SA, Neuhausen SL, Lynch HT, Weber BL. Breast
   cancer risk after bilateral prophylactic oophorectomy in BRCA1
   mutation carriers. J Natl Cancer Inst 1999; 91: 1475-1479.
9. Metcalfe K, Lynch HT, Ghadirian P, Tung N, Olivatto I, Warner
   E, Olopade OI, Eisen A, Weber B, McLennan J, Sun P, Foulkes
   WD, Narod SA. Contralateral breast cancer in BRCA1 and
   BRCA2 mutation carriers. J Clin Oncol 2004; 22: 2328-2335.
10. Narod SA, Brunet JS, Gazdar A, Robson M, Heimdal K, Neuhausen
    SL, Stoppa-Lyonnet D, Lerman C, Pasini B, de los
    Rios P, Weber B, Lynch H; Hereditary Breast Cancer Clinical

Table 13. Percentage of women who undertook preventive activities in
the year preceding the questionnaire

| Preventive activity | BRCA1+ (n = 63) | BRCA1- (n = 99) |
|--------------------|-----------------|-----------------|
|                    | Family history+ | Family history- |
|                    | (n = 43)        | (n = 55)        |
| A. Prevention of breast cancer |
| Mammography2       | 71% 62% 54%     |                 |
| Self-examination (mean/year) | 13.4 11.6 7.5  |
| Palpation by physician | 68% 58% 50%     |
| Preventive mastectomy | 5% 0% 0%       |
| B. Prevention of ovarian cancer |
| Ovarian ultrasound | 67% 60% 39%     |                 |
| CA-125             | 56% 21% 9%      |                 |
| Preventive oophorectomy3 | 23% 2% 4%   |

1Family history+ – at least two cases of breast/ovarian cancer in maternal or
paternal line; 2 – women aged > 35 years; 3 – women aged > 40 years.
Study Group. Tamoxifen and risk of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers: a case-control study. Hereditary Breast Cancer Clinical Study Group. Lancet 2000; 356: 1876-1881.

11. Gorski B, Byrski T, Huzarski T, Jakubowska A, Menkiszak J, Gronwald J, Pluzanska A, Bebenek M, Fischer-Maliszewska L, Grzybowska E, Narod SA, Lubinski J. Founder mutations in the BRCA1 gene in Polish families with breast-ovarian cancer. Am J Hum Genet 2000; 66: 1963-1968.

12. Gorski B, Jakubowska A, Huzarski T, Byrski T, Gronwald J, Grzybowska E, Mackiewicz A, Stanwick M, Bebenek M, Sorakos D, Fischer-Maliszewska L, Haus O, Janiszewska H, Niepsuj S, Goczo S, Zaremba L, Posmyk M, Pluzanska M, Kilar E, Czudowska D, Wasko B, Miturski R, Kowalczyk JR, Urbanski K, Szwiec M, Koc J, Debnik A, Dziemianek A, Cebulska T, Cewalkska E, Toloczko-Grabarek A, Zajaczk S, Menkiszak J, Medrek K, Masej B, Mierzejewski M, Narod SA, Lubinski J. A high proportion of founder BRCA1 mutations in Polish breast cancer families. Int J Cancer 2011; 140: 683-688.

13. Grzybowska E, Sieminska M, Zientek H, Kalinowska E, Michalska J, Ustracza-Hulka B, Rogozinska-Szczepka J, Kazmierzczak-Maciejewska M. Germline mutations in the BRCA1 gene predisposing to breast and ovarian cancers in Upper Silesia population. Acta Biochim Pol 2002; 49: 351-356.

14. Jasinska A, Krzyzosiak WJ. Prevalence of BRCA1 founder mutations in western Poland. Hum Mutat 2001; 17: 75.

15. Perkowska M, Brozek I, Wysocka B, Haraldsson K, Sandberg T, Janiszewska H, Haus O, Lauda-Swieciak A, Pasinska M, Gersztenkorn A, Proniewska J, Batorowski J, Janiszewska H, Niepsuj S, Grzybowska E, Mackiewicz A, Chodakowska M, Zajaczk S, Menkiszak J, Medrek K, Masej B, Mierzejewski M, Narod SA, Lubinski J. A high proportion of founder BRCA1 mutations in Polish breast cancer families. Int J Cancer 2011; 140: 683-688.

16. Janiszewska H, Haus O, Lauda-Swieciak A, Pasinska M, Laskowski R, Szymanski W, Gorski B, Lubinski J. Frequency of three BRCA1 gene founder mutations in breast/ovarian cancer families from the Pomerania-Kujawy region of Poland. Clin Genet 2003; 64: 502-508.

17. King MC, Marks JH, Mandell JB, New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003; 302: 643-646.

18. Mosleh R, Chu W, Karlan B, Fishman D, Risch H, Fields A, Smolkin D, Ben-David Y, Rosenblatt J, Russo D, Schwartz P, Tung N, Warner E, Rosen B, Friedman J, Brunet JS, Narod SA. BRCA1 and BRCA2 mutation analysis of 208 Ashkenazi Jewish women with ovarian cancer. Am J Hum Genet 2000; 66: 1259-1272.

19. Warner E, Foulkes W, Goodwin P, Meschina W, Blondal J, Paterson C, Ozcelik H, Goss P, Allingham-Hawkins D, Hamel N, Di Prospero L, Contiga V, Serruya C, Klein M, Moslehi R, Honeyford J, Liede A, Glendon G, Brunet JS, Narod S, Johansson U, Sellberg G, Borg A, Liman J. BRCA1 and BRCA2 mutation analysis in breast-ovarian cancer families from northeastern Poland. Hum Mutat 2003; 21: 553-554.

20. Manoukian S, Eccles DM, Tang N, Olah E, Anton-Culver H, Rebbeck T, Neuhausen SL, Ghadirian P, Foulkes WD, Gershoni-Baruch R, Ainsworth P, Friedman E, Daly M, Garber JE, Karlan B, Olopade Ol, Tung N, Sam HL, Eisen A, Osborne M, Olsson H, Gilchrist D, Sun P, Narod SA. Agre at menarche and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Cancer Causes Control 2005; 16: 667-674.

21. Lipworth L, Bailey IL, Truly-Holmes D. History of breast-feeding in relation to breast cancer risk: a review of the epidemiologic literature. J Natl Cancer Inst 2000; 92: 302-312.

22. Jernström H, Lubinski J, Lynch HT, Ghadirian P, Neuhausen S, Isaacas C, Weber BL, Horsman D, Rosen B, Foulkes WD, Friedman E, Gershoni-Baruch R, Ainsworth P, Daly M, Garber JE, Olsson H, Sun P, Narod SA. Breast-feeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst 2004; 96: 1094-1098.

23. Ghadirian P, Lubinski J, Lynch HT, Neuhausen S, Isaacas C, Weber BL, Horsman D, Rosen B, Foulkes WD, Friedman E, Gershoni-Baruch R, Ainsworth P, Daly M, Garber JE, Olsson H, Sun P, Narod SA. Breast-feeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst 2004; 96: 1094-1098.

24. Gronwald J, Horsman D, Lubinski J, Lynch HT, Neuhausen S, Isaacas C, Weber BL, Pearson B, Sun P, Narod SA. Smoking and the risk of breast cancer among carriers of BRCA mutations. Int J Cancer 2011; 140: 413-416.

25. Nkondjock A, Ghadirian P, Kotsopoulos J, Lubinski J, Lynch H, Kim-Sing C, Horsman D, Rosen B, Isaacas C, Weber B, Foulkes W, Ainsworth P, Tung N, Eisen A, Friedman E, Eng C, Sun P, Narod SA. Coffee consumption and breast cancer risk among BRCA1 and BRCA2 mutation carriers. Int J Cancer 2006; 118: 103-107.

26. Grzybowska E, Huzarski T, Byrski B, Medrek K, Menkiszak J, Monteiro AN, Sun P, Lubinski J, Narod SA. Cancer risks in first degree relatives of BRCA1 mutation carriers: effects of mutation and proband disease status. J Med Genet 2006; 43: 424-428.

27. Antoniou AC, Pharoah PD, Narod S, Risch HA, Eeetram JE, Hopper JL, Olsson H, Johannsson B, Borg A, Pasini B, Radice P, Manoukian S, Eccles DM, Tang N, Olah E, Anton-Culver H, Warner E, Lubinski J, Gronwald J, Gorski B, Tung N, Rahiomin M, Thorlacius S, Eerola H, Nevanlinna H, Syrjakoski K, Kallioniemi OP, Thompson D, Evans C, Peto J, Lalloo F, Evans DG, Easton DF. Breast and ovarian cancer risk to female offspring of BRCA1 carriers. Int J Cancer 2005; 117: 1911-1915.
different approaches and different biases. Int J Cancer 2005; 113: 166-167.

36. Evans DG, Shenton A, Sharif S, Woodward E, Laloo F, Maher ER. Non-random transmission of mutant alleles to female offspring in BRCA carriers. J Med Genet 2005; 42: e6.

37. Ganesan S, Silver DP, Greenberg RA, Avni D, Drapkin R, Miron A, Mok SC, Randharianan V, Brodie S, Salstrom J, Rasmussen TP, Klimke A, Marrere C, Marahrens Y, Deng CX, Feunteun J, Livingston DM. BRCA1 supports XIST RNA concentration on the inactive X chromosome. Cell 2002; 111: 393-405.

38. Lee JT. Homozygous Tsix mutant mice reveal a sex-ratio distortion and revert to random X-inactivation. Nat Genet 2002; 32: 195-200.

39. Buller RE, Sood AK, Lallas T, Buekers T, Skilling JS. Association between nonrandom X-chromosome inactivation and BRCA1 mutation in germline DNA of patients with ovarian cancer. J Natl Cancer Inst 1999; 91: 339-346.

40. de la Hoya M, Fernandez JM, Tosar A, Godino J, Sanchez de Abajo A, Vidart JA, Perez-Segura P, Diaz-Rubio E, Caldes T. Association between BRCA1 mutations and ratio of female to male births in offspring of families with breast cancer, ovarian cancer, or both. JAMA 2003; 290: 929-931.

41. Feunteun J, Champet A, Helbling-Leclerc A, Stoppa-Lyonnet D, Belotti M, Nogues C, Bonaiti-Pellie C. Sex ratio among the offspring of BRCA mutation carriers. JAMA 2004; 292: 687-688.

42. Katar K, Brunet JS, Moller P, Hugel L, Warner E, McLaughlin J, Wong N, Narod SA, Foulkes WD. Ratio of female to male offspring of women tested for BRCA1 and BRCA2 mutations. J Med Genet 2004; 41: e103.

43. Balmana J, Diez O, Campos B, Majewski M, Soru J, Alonso C, Boiget M, Garber JE. Sex ratio distortion in offspring of families with BRCA1 or BRCA2 mutant alleles: an ascertainment bias phenomenon? Breast Cancer Res Treat 2005; 92: 273-277.

44. Chenevix-Trench G, Sinilnikova OM, Suthers G, Pandeya N, Mazoyer S, Sambrook JF, Goldup S, Goldgar D, Lynch HT, Lenoir GM, Cheetham G; kConFab. Ratio of male to female births in the offspring of BRCA1 and BRCA2 carriers. Fam Cancer 2005; 4: 73-75.

45. Mealiffe ME. Sex ratios in families with BRCA mutations. JAMA 2003; 290: 2544.

46. Polish Main Statistics Office. Available at: http://www.gus.pl (13.05.2005).

47. Gronwald J, Tung N, Foulkes WD, Offit K, Gershoni R, Daly M, Kim-Sing C, Olsson H, Ainsworth P, Eisen A, Saal H, Friedman E, Olopade O, Osborne M, Weitzel J, Lynch H, Ghadirian P, Lubinski J, Sun P, Narod SA; Hereditary Breast Cancer Clinical Study Group. Tamoxifen and contralateral breast cancer in BRCA1 and BRCA2 carriers: an update. Int J Cancer 2006; 118: 2281-2284.

48. Mouchawar J, Hensley-Alford S, Laurion S, Ellis J, Kulchak-Rahm A, Finucane ML, Meenan R, Axell L, Pollack R, Ritzwoller D. Impact of direct-to-consumer advertising for hereditary breast cancer testing on genetic services at a managed care organization: a naturally-occurring experiment. Genet Med 2005; 7: 191-197.