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BRCA2 mutation carriers, reproductive factors and breast cancer risk

Laufey Tryggvadottir[1], Elinborg J Olafsdottir[1], Sigfridur Gudlaugsdottir[2], Steinunn Thorlacius[2]*, Jon G Jonasson[1,3], Hrafn Tulinius[1] and Jorunn E Eyfjord[2,3]

[1]Icelandic Cancer Registry, Reykjavik, Iceland
[2]Molecular and Cell Biology Laboratory of the Icelandic Cancer Society, Reykjavík, Iceland
[3]Faculty of Medicine, University of Iceland, Reykjavik, Iceland
*Current address: Iceland Genomics Corporation, Reykjavik, Iceland

Correspondence: Laufey Tryggvadóttir (e-mail: laufeyt@krabb.is)

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Abstract

Background Germline mutations in the BRCA genes dramatically increase the risk of breast cancer. In the general population, breast cancer risk is affected by age at menarche, by age at first birth, by the number of births and by the duration of breast feeding. Whether this is true for mutation carriers is not clear.

Methods In a case–control study, nested in a population-based cohort of the Icelandic Cancer Detection Clinic, two groups of cases were defined, matched on year of birth, on age at diagnosis and on age when giving information on reproductive factors: 100 carriers of the Icelandic founder *BRCA2* mutation 999del5, and 361 *BRCA2*-negative cases. The mean age at diagnosis was 48 years. There were 1000 women in a matched group of unaffected controls. Conditional logistic regression was used for the analysis.

Results An increased number of births was associated with a decreased risk of breast cancer in *BRCA2*-negative cases but not in *BRCA2*-positive cases. A negative association between risk and duration of breast feeding was observed only in the mutation carriers. These associations were not statistically significant, but the effects of the two variables differed significantly according to mutation status ($P = 0.007$ and $P = 0.045$ for interaction with number of births and with duration of breast feeding, respectively). This was maintained when limiting the analysis to women diagnosed older than the age of 40 years.

Conclusion The association between breast cancer and the number of pregnancies and between breast cancer and the duration of breast feeding was not the same for carriers and noncarriers of a detrimental *BRCA2* mutation. In the context of other epidemiological and laboratory studies, this may indicate that the product of the *BRCA2* gene has a function relating to the differentiation of epithelial tissue in the breast.

Keywords: *BRCA2*, breast cancer, cohort study, risk factors

Introduction

Mutations in the *BRCA1* and *BRCA2* genes dramatically increase the risk of breast cancer. Numerous mutations in each gene have been found in most populations studied, but only one mutation in each gene has been identified to date in the Icelandic population of 285,000; a rare mutation in the *BRCA1* gene, and a much more frequent mutation in the *BRCA2* gene. The *BRCA2* mutation (999del5) is present in 7–8% of unselected breast cancer patients in Iceland [1–3], and it has a much higher prevalence (24%) in women diagnosed younger than 40 years of age [2]. The mutation explains around 40% of the increased breast cancer risk in first-degree relatives of Icelandic breast cancer patients and all excess risk of prostate cancer and ovarian cancer in relatives of breast cancer patients [4]. The estimated breast cancer risk in mutation carriers at the age of 70 years is 37–44% [4,5].

The two *BRCA* genes code for large proteins that are involved in basic cellular functions such as DNA repair,

CDC = Cancer Detection Clinic; PCR = polymerase chain reaction.

cell cycle control and transcription [6]. Why the effects of mutations in these genes are mainly specific for cancers that are hormonally related, such as breast cancer, ovarian cancer and prostate cancer, is presently an important unanswered question. There are indications that the product of the *BRCA1* gene has a role in oestrogen-receptor signalling [7], and recent results suggest that the *BRCA2* gene may have a function relating to terminal differentiation of breast epithelial cells [8].

An increasing age at menarche, a low age at first birth, an increasing parity and breast feeding are associated with a reduced risk of breast cancer in the general population [9–12]. However, after each pregnancy there is a transient increase in the risk of breast cancer [13–15]. The strength and direction of the effects of pregnancies are therefore related to time since last birth, and thus present differently according to whether the women studied are at a fertile age [12]. Furthermore, these associations may be affected by a disrupted function of either of the *BRCA* genes [16–20] but the picture emerging from the few studies published to date is still far from being clear. Further studies in this field are important as they may increase our understanding of the function of the *BRCA* genes, as well as the effects of modifying factors on the risk in mutation carriers.

Familial breast cancer has been extensively studied at the Icelandic Cancer Society for more than two decades [1,2,4,5,21–29]. The present study used information on 100 breast cancer cases carrying the Icelandic *BRCA2* mutation, who were compared with breast cancer cases without the mutation and with unaffected controls. All participants had contributed information on reproductive risk factors, before diagnosis of the cases, in the cohort study of the Cancer Detection Clinic (CDC) of the Icelandic Cancer Society. The hypothesis of the present study was that the associations between breast cancer and age at menarche, pregnancies and breast feeding would be different for women with and without the mutation.

Materials and methods

The design was a nested case–control study. Information on reproductive factors for all participants was obtained from the CDC cohort, where most Icelandic women 20 years or older have given answers to questions on potential risk factors for breast cancer. These data have been collected as a part of the nationwide, centralized cervical cancer screening programme that started in 1964 and later also as a part of the nationwide breast cancer screening that started in 1987. The questions have changed with time; for example, information on breast feeding has only been collected since 1979. The databank is described in more detail elsewhere [12,30,31].

The number of women in the cohort to date is 98,000. A large proportion of the women have responded to the questionnaire on more than one occasion. The information used in the present study was that given at the last visit before diagnosis of the *BRCA2*-positive cases (and the same year for the matched *BRCA2*-negative cases and matched controls). There was a possibility of supplementing missing or invalid items with information given at other visits, concerning events that had evidently already occurred at the last visit before diagnosis. However, analyses on data corrected in this way showed that the information for controls became considerably more complete than for the cases, although the analyses were performed blindly with respect to case–control status. This was because the average number of visits to the CDC was lower after the date of diagnosis for cases than for their matched controls, which is probably due to their lower life expectancy. Since this fact might have introduced a bias, uncorrected information given at the last visit before diagnosis was used. Variables used in the present analysis were age at menarche, age at first birth, number of births and breast feeding.

All participants in the study, two case groups and controls, belonged to the CDC cohort. Only information given before diagnosis was used for the breast cancer cases.

The group of *BRCA2*-positive cases was defined using information on mutation status for breast cancer patients who had participated in studies of the Icelandic Cancer Society that included collection of blood or paraffin samples (see a further description later). The number of mutation carriers with invasive breast cancer that had been identified in those studies before closure of the present study was 145. Using precoded personal identifiers, record linkage with the CDC cohort resulted in 142 *BRCA2*-positive cases who had ever contributed information as a part of the CDC cohort. Of these cases, 39 women were excluded because they had only given information after diagnosis of breast cancer. For three of the remaining 103 mutation carriers it was not possible to find a matching case (see later), leaving 100 mutation carriers for the analysis. They were diagnosed in the years 1967–2001.

When defining the matched group of *BRCA2*-negative cases, we started with all Icelandic women diagnosed in the period January 1965 to December 2001 (3290 women). Record linkage with the CDC cohort resulted in 2679 cases or 81% of all women diagnosed in the period. After exclusion of women who had only given information after diagnosis, we sought four cases from this group, individually matched to each of the *BRCA2*-positive cases on year of birth, on year of diagnosis and on year when giving information on reproductive factors (±3 years). A total of 1572 women fulfilled the criteria, but as only four *BRCA2*-negative cases per mutation carrier were sought and since for some of the 100 mutation carriers less than

four matching women were found, this resulted in a group of 361 *BRCA2*-negative cases that best matched the 100 mutation carriers. Of those cases 286 had been tested for mutation status, leaving 75 cases with unknown status (because they had not been participants in the later-described studies). However, it is probably justified to refer to the group as *BRCA2*-negative because, from the earlier-defined subgroup of (1572 + 100) Icelandic breast cancer cases, the 100 mutation carriers had already been removed. Since 7–8% of Icelandic breast cancer patients carry the *BRCA2* founder mutation, around 125 positive cases were expected from this subgroup. However, 100 of those positive cases had already been removed so, among the remaining 1572 women, only 25 carriers are expected (1.6%). Therefore, only one or two mutation carriers are expected among the 75 cases with unknown status.

The control group of 1000 unaffected women was drawn from the CDC cohort with individual matching to the *BRCA2*-positive cases on year of birth and on year when contributing information. The controls had to have been alive when their matched mutation carrier was diagnosed.

It could be of concern that the *BRCA2*-positive cases might be a selected group with respect to family history, since they were participants in earlier studies of the Icelandic Cancer Society. However, the majority of the group of 100 *BRCA2*-positive cases (81 women) came from an ongoing investigation inviting all cases who are alive in Iceland. These cases are thus unselected with respect to family history. An additional six women belonged to an unselected group of breast cancer cases recruited in a study on p53 mutations and risk factors using paraffin-embedded samples. The 13 remaining women had participated in earlier studies on family history of breast cancer. To check whether the inclusion of these cases could have introduced a bias, a separate analysis was performed excluding those 13 women.

The unaffected controls were not tested for mutation status. The prevalence of the *BRCA2* mutation 999del5 in the general population is around 0.6% [2] so only six positive women were expected to be among the 1000 controls.

Odds ratios and 95% confidence intervals were estimated applying conditional logistic regression [32]. The risk associated with age at menarche, with parity, with age at first birth and with total duration of breast feeding was estimated separately for the *BRCA2*-positive cases and for the *BRCA2*-negative cases, comparing them with controls, using conditional logistic regression. This comparison is not likely to be invalidated by the lack of information on mutation status in the controls since so few positive controls were expected. Furthermore, since it was not practical to try to estimate the effects of reproductive factors in a carrier group of six positive individuals among the 1000 controls, interaction between the reproductive factors and the *BRCA2* mutation status had to be tested using a case-only approach [33] where the *BRCA2*-negative cases were compared with the *BRCA2*-positive cases. Interaction was assumed to be present for a reproductive variable if the odds ratio for that variable differed significantly from 1.0. The analyses were performed using STATA Statistical Software [34].

Screening for the *BRCA2* 999del5 germline mutation was performed by PCR amplification and electrophoresis as previously described [1]. All analyses were carried out on precoded material and in full accordance with the requirements of the Icelandic Data Protection Authority and The National Bioethics Committee.

Results

The mean ages at diagnosis among *BRCA2*-positive cases and *BRCA2*-negative cases were 48.4 years (range 30–77 years) and 49.4 years (range 28–79 years), respectively. The mean ages when the *BRCA2*-positive cases, *BRCA2*-negative cases and controls had contributed information in the cohort study were 42.8 years (range 21–69 years), 43.3 years (range 20–69 years) and 42.8 years (range 21–69 years), respectively. The women were all born between 1912 and 1963 (median year of birth was 1939), and the cases were diagnosed in the years 1966–2001.

Table 1 compares the three groups of participants with respect to distribution of age at menarche and reproductive variables. Also presented are percentages with recorded responses for each item. The response rates are similar for the three groups. They are lowest for duration of breast feeding, due to the fact that registration of this variable first started in 1979. The *BRCA2*-positive cases were more likely than the controls to have given five or more births, whereas the *BRCA2*-negative cases had fewer births than the controls, as expected. The average total duration of breast feeding was lowest in the mutation carriers and was highest in the control group. The results did not allow for inferences with respect to whether this could be explained by problems with breast feeding in the mutation carriers. A very short total duration (less than 4 weeks) or no breast feeding was less common among parous *BRCA2*-positive cases than among noncarriers and controls, with percentages of 3.4%, 8.1% and 8.8%, respectively. The shorter average total duration of breast feeding in mutation carriers than in controls was mainly due to a lower percentage with overall duration of breast feeding for all children exceeding 2 years. When considering breast feeding for each child (data not shown), the percentages with duration of 4 weeks or less were similar in the mutation carriers and in controls (20.7%, 16.4% and 21.7% for *BRCA2*-positive cases, noncarriers and controls, respectively). However, *BRCA2*-positive cases

Table 1

Comparison of age at menarche and reproductive factors between *BRCA2*-positive cases, *BRCA2*-negative cases and controls

	BRCA2-positive cases ($n = 100$)		*BRCA2*-negative cases ($n = 361$)		Matched controls ($n = 1000$)	
Risk factor	% distribution	Mean	% distribution	Mean	% distribution	Mean
Age at menarche	$n = 85$ (85%)*		$n = 313$ (87%)*		$n = 860$ (86%)*	
< 13 years	25.9	13.2 years	31.3	13.1 years	27.4	13.3 years
13 years	35.3		29.1		28.9	
> 13 years	38.8		39.6		43.6	
Parous	$n = 99$ (99%)		$n = 355$ (98%)		$n = 991$ (99%)	
Yes	89.9		88.2		93.0	
No	10.0		11.8		7.0	
Parous women						
Age at first birth	$n = 85$ (96%)		$n = 300$ (96%)		$n = 880$ (95%)	
< 20 years	16.5	22.2 years	22.3	23.0 years	25.0	22.3 years
20–29 years	80.0		69.3		69.6	
> 29 years	3.5		8.3		5.4	
Number of births	$n = 89$ (100%)		$n = 313$ (100%)		$n = 922$ (100%)	
1–2	32.6	3.3 births	40.9	3.0 births	39.7	3.2 births
3–4	46.1		45.7		42.5	
5+	21.3		13.4		17.8	
Total duration of breastfeeding	$n = 58$ (65%)		$n = 209$ (67%)		$n = 640$ (69%)	
0 months	1.7	8.5 months	2.9	9.2 months	1.9	9.3 months
< 1 months	1.7		5.3		6.9	
1–6 months	44.8		37.3		38.4	
7–12 months	29.3		31.1		28.8	
13–24 months	19.0		18.7		17.2	
> 24 months	3.4		4.8		6.9	

*Percentages with recorded responses for each item.

were less likely than controls to have breast fed each child for longer than 6 months, with percentages being 5.2%, 12.0% and 11.9% for the affected mutation carriers, for the cases without the mutation and for the controls, respectively.

Table 2 presents the results from the multivariate analysis for parous women. The effects of age at menarche and of age at first birth were in the same direction for women with and without the mutation, and interaction with the mutation status was not statistically significant for these variables. On the contrary, each additional birth was associated with a nonsignificantly increased breast cancer risk (17%) among the mutation carriers, whereas a marginally significant 13% decrease in risk was observed for women without the mutation. Interaction was present between the mutation status and the number of births and between the mutation status and the total duration of breast feeding ($P = 0.007$ and $P = 0.045$, respectively). When limiting the analysis to cases diagnosed at age 40 years or older, the interaction was still present ($P = 0.005$ and $P = 0.045$ for the number of births and for the duration of breast feeding, respectively). Furthermore, after exclusion of 13 mutation carriers who had been recruited because of family history, the interaction remained statistically significant ($P = 0.005$ and $P = 0.021$ for the number of births and for the duration of breast feeding, respectively).

Discussion

The results indicate that there are differences between carriers and noncarriers of a detrimental mutation in the *BRCA2* gene with respect to the association between breast cancer risk and the number of pregnancies and between breast cancer risk and the total duration of breast

Table 2

Parous women: multivariate analysis of the effects of risk factors for *BRCA2*-positive cases and *BRCA2*-negative cases separately

	BRCA2-positive cases ($n = 100$) versus matched controls ($n = 1000$)		*BRCA2*-negative cases ($n = 361$) versus matched controls ($n = 1000$)	
Risk factor	Odds ratio (95% confidence interval)	*P* value	Odds ratio (95% confidence interval)	*P* value
Age at menarche	0.92 (0.74–1.14)	0.430	0.87 (0.77–0.99)	0.030
Age at first birth	1.01 (0.93–1.10)	0.738	1.02 (0.97–1.07)	0.352
Number of births	1.17 (0.95–1.43)*	0.132	0.87 (0.75–1.00)*	0.057
Total duration of breast-feeding (unit, 6 months)	0.96 (0.91–1.01)**	0.141	1.01 (0.98–1.03)**	0.579

Odds ratios are presented per unit increment of the variables. * $P = 0.007$, odds ratio = 1.49 for the interaction between the number of births and the mutation status. ** $P = 0.045$, odds ratio = 0.93 for the interaction between the total duration of breast feeding and the mutation status. When testing for interaction between the other variables and the mutation status, $P > 0.4$.

feeding. No difference was detected with respect to age at menarche and to age at first birth.

Only relatively few studies published to date have addressed the effects of breast cancer risk factors on *BRCA* mutation carriers [7,16–19,35–39]. The majority of these have indicated that mutation carriers respond in a special way to breast cancer risk factors. Johannsson and colleagues [19] found an increased risk of pregnancy-associated breast cancer in carriers of *BRCA1* mutations, and a weaker risk in carriers of *BRCA2* mutations. Jernstrom and colleagues [16] found indications that *BRCA1* mutation carriers were more likely to have problems with breast feeding than expected. Jernstrom and colleagues also reported [38] a positive association between breast cancer risk and an increasing number of births in *BRCA1* and *BRCA2* mutation carriers. This could, however, have been related to the low age of the cases in the study (≤40 years at diagnosis), and thus could be explained by the transient increase in risk following pregnancy that is also seen in the general population [13]. Hartge and colleagues [18] found that mothers of carriers of *BRCA1* and *BRCA2* mutations did not show the expected risk reduction associated with lower age at first birth and with an increasing number of births. In the recent study of Narod and colleagues [39] there was an increase in breast cancer risk in *BRCA1* mutation carriers associated with use of oral contraceptives, especially at young ages. No association was present between breast cancer risk and parity in this large group of *BRCA1* and *BRCA2* mutation carriers.

The present study has the advantage of using prospective information on risk factors from a population-based cohort for *BRCA2*-positive cases, for *BRCA2*-negative cases and for unaffected controls. The study group was not restricted to young cases; the median age at diagnosis was 48.4 years (range 30–77 years) and the interaction between mutation status and number of pregnancies persisted after excluding cases diagnosed younger than the age of 40 years. We believe that matching on birth year and age when responding reduced confounding and random variation due to changes with time in reproductive and breast feeding habits [30]. A possible bias due to inclusion of 13 *BRCA2*-positive cases who were recruited because of family history was ruled out, since the exclusion of this subgroup did not affect the observed interaction between the mutation status and the number of births. The present study included only carriers of one specific *BRCA2* founder mutation. However, it is probable that inferences can be drawn for carriers of other types of detrimental *BRCA2* mutations since this mutation is a 5 base pair deletion in exon 9 leading to an early truncation of the BRCA2 protein. On the contrary, this study provides no information regarding carriers of *BRCA1* mutations. The case groups were well defined but small, and thus there was relatively low power to detect the low relative risks associated with the effects of the reproductive variables according to earlier studies using the CDC cohort [9,10,12,30].

The lack of a significant positive association between breast cancer and age at first birth in the *BRCA2*-negative cases was rather unexpected since such a relationship has been confirmed in other studies based on the CDC cohort. However, the relationship has been found to be strongest in premenopausal cases [10,12]. The present lack of association could thus be explained by a low power to detect the association because there were only 245 cases in the *BRCA2*-negative group who were younger than age 55 years at diagnosis. Decreased breast cancer risk associated with breast feeding was mainly confined to young cases in a recent Icelandic study [30]. In accordance with the present results this might be related to the fact that 24% of breast cancer patients diagnosed younger than age 40 years in Iceland are carriers of the *BRCA2* founder mutation [2].

Much is still unclear about the roles of the *BRCA* gene products in the biology of epithelial tissue, and explanations of the tissue specificity of the effects of mutations are lacking [40]. It is known, however, that the BRCA proteins are involved in basic cellular functions such as DNA repair, cell cycle control and transcription. More specifically, there are suggestions that the *BRCA1* gene has a role in steroid hormone signalling [6,7]. Studies of the murine *brca1* gene indicate that it is involved in the process of proliferation and differentiation in response to ovarian hormones [41].

Support for a role of the *BRCA1* gene relating to differentiation of breast tissue in humans comes from the work of Russo and colleagues. They have investigated the effects of pregnancies on the differentiation of epithelial cells in the mammary gland, both in animal models and in human breast tissue [42–45]. The results of Russo and colleagues underline the importance of undifferentiated cells in breast carcinogenesis, demonstrating their susceptibility to the effects of carcinogens contrary to differentiated structures that are less susceptible. Furthermore, Russo and colleagues have shown that, during the lifespan of a female, the breast tissue undergoes complicated developmental changes, and that parous women have generally much more complex lobular structures (lobules type 3) than nullipara (lobules type 1). In a recent study, Russo and colleagues' results indicated that women with a family history of breast cancer or with *BRCA1* mutations have much less pronounced changes related to pregnancy, suggesting that the *BRCA1* gene may have a functional role in the branching pattern of the breast during lobular development [46].

Results of a recent study from our laboratory suggest that the product of not only the *BRCA1* gene, but also the *BRCA2* gene may have a role in the differentiation of breast epithelial cells [8]. The protein Stat5a belongs to a family of transcription factors that mediate the transcriptional response to a diverse group of cytokines and growth factors. Stat5a (mammary gland factor), which is essential for growth and terminal differentiation of breast epithelial cells and is strongly activated towards the end of pregnancy, was shown to form a complex with BRCA1 and BRCA2 proteins in breast epithelial cells upon stimulation with prolactin. Furthermore, the effects of Stat5a were modulated by both BRCA1 and BRCA2 proteins.

The hypothesis of the present study was that the association between breast cancer and age at menarche, pregnancies and breast feeding differed between women with and without the mutation. The hypothesis was supported for the number of pregnancies and the duration of breast feeding. A case-only design was used for estimating the interaction between reproductive variables and the *BRCA2* mutation status. The validity of this approach has been shown to be dependent on the assumption of independence between the environmental and genetic factor [47].

In this study it was not possible to verify this assumption. We could not check whether the number of children or the duration of breast feeding was associated with the mutation status because the prevalence of the mutation is very rare in the population [2] and only six carriers were expected among the controls. The interpretation of the results could therefore either be that hormones associated with pregnancies and with breast feeding affect breast cancer risk differently in mutation carriers and in noncarriers, or alternatively that mutation carriers are more likely to have an increased number of children (i.e. be more fertile) than other women and are more likely to present with a short duration of breast feeding due to an inherited reduced ability to breast feed. Even though increased fertility of the mutation carriers cannot be ruled out as an explanation for the observed association, the study by Russo and colleagues [46] supports a function of the *BRCA1* gene in the process of differentiation as a response to ovarian hormones. The results cited earlier, indicating that the product of the *BRCA2* gene might be important in the process of growth and terminal differentiation of breast epithelial cells, support that the same could be true for the *BRCA2* gene.

With respect to potential problems with milk production of mutation carriers, there has been one report indicating that this could be true [16]. In the present study, information was not available relating to potential problems with breast feeding. The mutation carriers were just as likely to have ever breast fed as the other two groups, and a very short duration of breast feeding each child was as common among the mutation carriers as among the controls. The observed lower mean duration of lactation among the mutation carriers was due to lack of breast feeding for more than 6 months per child. The present results do not allow inferences to be drawn, with respect to potential problems with breast feeding.

Conclusion

The association between breast cancer risk and the number of pregnancies and between breast cancer risk and the duration of breast feeding differed among women with and without the *BRCA2* mutation. The present study does not allow for differentiating between effect modification by mutation status on the one hand and inherited differences with respect to fertility or problems with breast feeding due to presence of the mutation on the other. Further studies are needed both on fertility and potential problems with breast feeding in unaffected mutation carriers, and on the effects of hormones related to pregnancy and breast feeding with respect to signalling and differentiation in breast epithelial cells of mutation carriers. In the context of current knowledge, however, the results are

supportive of a hypothesis stating that the product of the *BRCA2* gene has a function relating to the differentiation of epithelial tissue in the breast in response to the hormones of pregnancy.

Competing interests

None declared.

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Correspondence

Laufey Tryggvadóttir, Icelandic Cancer Registry, Skógarhlíð 8, P.O. Box 5420, IS-125 Reykjavík, Iceland. Tel: +354 540 1900; fax: +354 540 1910; e-mail laufeyt@krabb.is