Cancer Causes and Control (2005) 16:667–674
DOI 10.1007/s10552-005-1724-1


# Age at menarche and the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers

Joanne Kotsopoulos[1,2], Jan Lubinski[3], Henry T. Lynch[4], Susan L. Neuhausen[5], Parviz Ghadirian[6], Claudine Isaacs[7], Barbara Weber[8], Charmaine Kim-Sing[9], William D. Foulkes[10], Ruth Gershoni-Baruch[11], Peter Ainsworth[12], Eitan Friedman[13], Mary Daly[14], Judy E. Garber[15], Beth Karlan[16], Olufunmilayo I. Olopade[17], Nadine Tung[18], Howard M. Saal[19], Andrea Eisen[20], Michael Osborne[21], Hakan Olsson[22], Dawna Gilchrist[23], Ping Sun[1] & Steven A. Narod[1,*]

[1]*Centre for Research in Women's Health, University of Toronto, 790 Bay Street, 7th Floor, Women's College Hospital, Canada;* [2]*Department of Nutritional Sciences, University of Toronto, Ontario, Canada;* [3]*Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland;* [4]*Department of Preventive Medicine and Public Health, Creighton University School of Medicine, Omaha, NE, USA;* [5]*Epidemiology Division, Department of Medicine, University of California, Irvine, USA;* [6]*Epidemiology Research Unit, Research Centre, Centre Hospitalier de l' Universitaire Montréal, CHUM Hôtel Dieu, Département de Nutrition, Faculte du Medicine, Quebec, Canada;* [7]*Lombardi Cancer Center, Georgetown University Medical Center, Washington, USA;* [8]*British Columbia Cancer Agency, Vancouver, BC, Canada;* [9]*Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, PA, USA;* [10]*Departments of Medicine, Human Genetics, and Oncology, McGill University, Montréal, QC, Canada;* [11]*Institute of Genetics, Rambam Medical Center, Haifa, Israel;* [12]*London Regional Cancer Center, London, ON, Canada;* [13]*The Suzanne Levy Gertner Oncogenetics Unit, The Chaim Sheba Medical Center, Tel-Hashomer, Israel;* [14]*Division of Population Science, Fox Chase Cancer Center, Philadelphia, PA, USA;* [15]*Dana Farber Cancer Institute, Boston, MA, USA;* [16]*Gynecology Oncology, Cedars Sinai Medical Center, Los Angeles, CA;* [17]*Center for Clinical Cancer Genetics, University of Chicago, Chicago, IL, USA;* [18]*Beth Isreal Medical Center, Boston, MA, USA;* [19]*Hereditary Cancer Program, Division of Human Genetics, Children's Hospital Medical Center, Cincinnati, OH, USA;* [20]*Toronto-Sunnybrook Regional Cancer Center, Toronto, ON, Canada;* [21]*Strang Cancer Prevention Center, New York, NY, USA;* [22]*Jubileum Institute, Department of Oncology, Lund University Hospital, Lund, Sweden;* [23]*Internal Medicine/Medical Genetics, WCM University of Alberta, Edmonton, AB, Canada*

Received 17 December 2004; accepted in revised form 03 February 2005

*Key words:* *BRCA1*, *BRCA2*, age at menarche, breast cancer, case–control study, reproductive modifiers.

## Abstract

Age at menarche is a strong and consistent predictor of breast cancer risk in the general population, but has not been well studied in women with a family history of breast cancer. We conducted this study to examine whether the presence of a deleterious *BRCA1* or *BRCA2* mutation influences age at menarche and to investigate whether or not there is an association between age at menarche and the risk of breast cancer in *BRCA1* or *BRCA2* mutation carriers. The presence of a deleterious *BRCA1* or *BRCA2* mutation did not appear to influence a woman's age at menarche. A matched case–control study was conducted on 1311 pairs of women who have been identified to be carriers of a deleterious mutation in either the *BRCA1* (n = 945 pairs) or the *BRCA2* gene (n = 366 pairs). Information about age at menarche was derived from a questionnaire routinely administered to carriers of a mutation in either gene. Among women who carried a deleterious *BRCA1* mutation, age at menarche was inversely associated with the risk of breast cancer ($p$ trend = 0.0002). This

* Address correspondence to: Steven A. Narod, Centre for Research in Women's Health, University of Toronto, 790 Bay Street, Room 750, 7th Floor, Women's College Hospital, Toronto, Ontario, M5G 1N8, Canada. Ph.: +1-416-351-3765; Fax: +1-416-351-3767; E-mail: steven.narod@swchsc.on.ca

association was not observed among *BRCA2* mutation carriers ($p$ trend = 0.49). Compared with *BRCA1* carriers whose age at menarche was ≤11 years, women with a menarcheal age between 14 and 15 years old had a 54% reduction in risk (OR = 0.46; 95% CI 0.30–0.69). This study implicates early age at menarche as a determinant of breast cancer among women with a *BRCA1* mutation.

## Introduction

In the general population, reproductive factors, including early age at menarche, late age at first full-term pregnancy, low parity, and late age at menopause are established risk factors for an increased risk of breast cancer [1, 2]. Oophorectomy prior to menopause protects against the development of breast cancer. The underlying mechanism is thought to relate to lifetime exposure to ovarian hormones, particularly estrogen [3]. Reproductive factors, including parity and breastfeeding, have also been reported to influence risk among women with a hereditary predisposition to breast cancer, but to date, age at menarche has not been well studied.

The age at menarche is falling throughout the world [4]. This decline is likely influenced by anthropometric measures, nutritional influences, and decreasing physical activity during childhood (reviewed in [5]). An early age at menarche has been shown to be a positive predictor of breast cancer risk in general, although no clear association has been found for women with a family history of breast cancer [6–8]. Whether or not there is a relationship between menarcheal age and breast cancer risk in women with a *BRCA1* or *BRCA2* mutation is not clear [9–11]. First, we examined whether the presence of a deleterious *BRCA1* or *BRCA2* mutation influences age at menarche. We then performed a matched case–control study to investigate whether or not there is an association between age at menarche and the risk of breast cancer in women with a deleterious *BRCA1* or *BRCA2* mutation.

## Materials and methods

Eligible study subjects included living women who were identified from 55 participating centers in eight countries. These women were participants in ongoing clinical research protocols at the host institutions. All study subjects (with the exception of those from the University of Utah) received counseling, provided written informed consent for genetic testing, and completed a questionnaire that asked for all relevant information regarding family history, reproductive and medical histories, and selected lifestyle factors including smoking and the use of oral contraceptives. Questionnaires were administered at the individual centers at the time of a clinic appointment or at their home at a later date. Additional variables of interest included information on demography and ethnic group.

The institutional review boards of the host institutions approved the study. In most cases, testing was initially offered to women who had been affected with breast or ovarian cancer. When a *BRCA1* or *BRCA2* mutation was identified in a proband or relative, genetic testing was offered to other at-risk women in the family. Mutation detection was performed using a range of techniques, but all nucleotide sequences were confirmed by direct sequencing of DNA. A woman was eligible for the current study when the molecular analysis established that she was a carrier of a deleterious mutation in the *BRCA1* or *BRCA2* gene. Most (>95%) of the mutations identified in the study subjects were either non-sense mutations, deletions, insertions, or small frameshifts.

The aim of the first part of the study was to examine whether mutation status influences age at menarche. Non-carriers were women who underwent genetic testing and were found to not be carriers of a deleterious *BRCA1* or *BRCA2* mutation. These women were from families where a mutation had previously been identified, and who underwent genetic testing at the Centre for Research in Women's Health, and were found not to carry the family mutation. Since information was not available for European controls, we limited this analysis to Canadian and American women. Potential subjects were excluded if information regarding age at menarche or mutation status was missing. After exclusion, there was a total of 3947 women available for the study, including 2107 *BRCA1* mutation carriers, 1053 *BRCA2* mutation carriers, and 787 non-carrier controls. The Student's $t$-test was used to compare the mean age at menarche between carriers and non-carriers.

In the second part of the study, a matched case–control analysis was carried out to test for a possible association between age at menarche and the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers. Information was available on cancer history for a total of 6133 women who carried a *BRCA1* or *BRCA2*

mutation. Potential subjects were excluded if they had been diagnosed with ovarian cancer (970 women), if information about age at menarche was missing (1261 women), or if information regarding mutation status (2 women), bilateral mastectomy (16 women) or oophorectomy (18 women) was missing. Control subjects were women who never had breast cancer and who were carriers of a mutation in the *BRCA1* or *BRCA2* gene. After exclusions, there was a total of 3866 eligible women, including 1896 women with breast cancer (potential case subjects) and 1970 women without breast cancer (potential controls).

A single control subject was selected for each case subject matched according to mutation in the same gene (*BRCA1* or *BRCA2*), year of birth (within three years), and the country of residence. Within Canada, French-Canadians were matched separately. In addition, the date of the interview, bilateral oophorectomy or bilateral mastectomy of the controls had to have occurred after the year of breast cancer diagnosis of the matched case subject. A total of 1311 matched case–control pairs was generated, including 945 pairs with *BRCA1* mutations and 366 pairs with *BRCA2* mutations.

Age at menarche was compared between the case subjects and control subjects within each strata using the Student's *t*-test. This test statistic was also used for all other continuous variables. One-way analysis of variance (ANOVA) was used to assess the difference in mean body mass index (BMI) (kg/m$^2$) at age 18 stratified by age at menarche in all case and control subjects. The multivariate odds ratios (OR), 95% confidence intervals (CI) and tests for linear trend were estimated by use of conditional logistic regression. A multivariate analysis was carried out to control for the potential confounding effects of oral contraceptive (OC) use, parity and BMI at age 18. OC use was coded as ever or never user; and parity was coded as zero, one, two, three, four, and four or more births. All statistical tests were two-sided. All analyses were performed using the SAS statistical package, version 8.1 (SAS Institute, Cary NC).

## Results

There was no significant difference in mean age at menarche in carriers and non-carriers from North America ($p = 0.97$) (Table 1). Case and control subjects were similar with regard to mutation status, country of residence, OC use, and mean parity (Table 2). Mean year of birth was slightly earlier in the case subjects than the control subjects (1953.5 *versus* 1954.4). Case subjects had an earlier age at menarche than the control subjects (12.7 *versus* 12.9 years; $p = 0.006$).

*Table 1.* Mean age at menarche of Canadian and American *BRCA* mutation carriers *versus* non-carriers

| | Mean age at menarche (range) | | |
|---|---|---|---|
| | Non-carriers | Carriers | *p* value[a] |
| All (*BRCA1* and *BRCA2*) | 12.7 (N = 787) | 12.7 (N = 3160) | 0.97 |
| *BRCA1* | 12.6 (N = 555) | 12.7 (N = 2107) | 0.53 |
| *BRCA2* | 12.8 (N = 232) | 12.7 (N = 1053) | 0.30 |

[a] All *p* values are univariate and were derived using the Student's *t*-test.

Among women who carried a deleterious *BRCA1* mutation, age at menarche was inversely associated with the risk of breast cancer ($p$ trend = 0.0002) (Table 3). Compared to women whose age at menarche was ≤11 years, women with a menarcheal age between 14 and 15 years old had a 54% (OR = 0.46; 95% CI 0.30–0.69) decrease in the risk of breast cancer (adjusting for parity, OC use and BMI). Although risk of breast cancer also appeared to decrease with later age at menarche among *BRCA2* mutation carriers, the effect did not reach statistical significance in this subgroup ($p$ trend = 0.49).

We adjusted for BMI in these comparisons because BMI is related both to age of menarche and to breast cancer risk. Because we did not have data on weight and height at age of menarche, BMI at age 18 was used. In Table 4A, it is shown that women with early age of menarche had, on average, a greater BMI at age 18 than those women with later age at menarche ($p < 0.0001$) and that BMI was negatively associated with breast cancer risk ($p = 0.05$).

## Discussion

The presence of a deleterious *BRCA1* or *BRCA2* mutation does not appear to influence a woman's age at menarche. Jernstrom *et al*. [10] also reported that age at menarche did not differ between 39 *BRCA1* and 11 *BRCA2* mutation carriers and compared to their relatives without mutations. In a recent study of carriers of the specific *BRCA2* Icelandic founder mutation (999del5), the authors reported no difference in the mean age at menarche between 100 *BRCA2*-positive cases, 361 *BRCA2*-negative cases and 1000 matched, unaffected, non-carrier controls [11].

We observed a statistically significant trend of decreasing breast cancer risk associated with age at

*Table 2.* Comparison of case and control subjects with *BRCA1* and *BRCA2* mutations

| Variable | Case subjects N = 1311 | Control subjects N = 1311 | *p* value[a] |
|---|---|---|---|
| Current age, mean ± SD[b] | 47.2 (10.2) | 45.5 (10.4) | < 0.0001 |
| Date of birth, mean year ± SD | 1953.5 (10.7) | 1954.4 (10.4) | < 0.0001 |
| Mutation, n (%) | | | |
| *BRCA1* | 945 (72.1) | 945 (72.1) | |
| *BRCA2* | 366 (27.9) | 366 (27.9) | |
| Parity[c], mean (SD) | 1.86 (1.3) | 1.90 (1.5) | 0.49 |
| Oral contraceptive use, No. (%) | | | |
| Ever | 716 (68.5) | 921 (70.7) | 0.21 |
| Age at menarche, mean (SD) | | | |
| *BRCA1* | 12.7 (1.4) | 13.0 (1.5) | 0.0007 |
| *BRCA2* | 12.7 (1.5) | 12.7 (1.5) | 0.86 |
| Age at first birth, mean (SD) | 25.1 (4.7) | 25.2 (4.7) | 0.62 |
| Country of residence[d], No. (%) | | | |
| United States | 559 (42.6) | 559 (42.6) | |
| Canada (excluding Quebec) | 314 (23.9) | 314 (23.9) | |
| Quebec | 124 (9.4) | 124 (9.4) | |
| United Kingdom | 9 (0.7) | 9 (0.7) | |
| Norway | 9 (0.7) | 9 (0.7) | |
| Sweden | 14 (1.0) | 14 (1.0) | |
| Austria | 4 (0.3) | 4 (0.3) | |
| Poland | 186 (14.2) | 186 (14.2) | |
| Israel | 92 (7.0) | 92 (7.0) | |

[a] All *p* values are univariate and were derived using the Student's *t*-test.
[b] SD = standard deviation.
[c] Parity includes live born and still born, and was included only in analysis if birth was one calendar year before the age of diagnosis of the matched case.
[d] Country of residence at time of testing.

*Table 3.* OR and 95% CI for breast cancer risk associated with age at menarche, stratified by *BRCA* mutation

| | Cases | Controls | OR[a] (95% CI) | *p* | *p* trend |
|---|---|---|---|---|---|
| *BRCA1* | | | | | |
| Age at menarche (years) | | | | | |
| (1) ≤11 | 173 | 127 | 1 | | |
| (2) 12 | 255 | 238 | 0.78(0.56–1.08) | 0.13 | |
| (3) 13 | 247 | 287 | 0.59(0.43–0.82) | 0.002 | |
| (4) 14 | 172 | 166 | 0.68(0.47–0.97) | 0.04 | |
| (5)≥15 | 98 | 127 | 0.46(0.30–0.69) | 0.0002 | |
| | | | | | 0.0002 |
| *BRCA2* | | | | | |
| Age at menarche (years) | | | | | |
| (1)≤11 | 69 | 56 | 1 | | |
| (2) 12 | 110 | 127 | 0.52 (0.31–0.88) | 0.01 | |
| (3) 13 | 89 | 97 | 0.61 (0.36–1.02) | 0.06 | |
| (4) 14 | 50 | 45 | 0.61 (0.32–1.17) | 0.13 | |
| (5)≥15 | 48 | 41 | 0.72 (0.37–1.38) | 0.32 | |
| | | | | | 0.49 |

[a] All ORs were derived using multivariate conditional logistic regression and were adjusted for OC use, parity and BMI at age 18.

menarche among women with a *BRCA1* mutation (*p* trend = 0.0002). For each year of menarcheal delay, there was an approximately 15% decrease in the risk of breast cancer. This protective effect was not observed among women carrying a deleterious *BRCA2* mutation (*p* trend = 0.49).

*Table 4A.* Comparison of mean BMI at age 18 according to age at menarche in all *BRCA* mutation carriers

| Age | All | Cases | Controls | *p* value[a] |
|---|---|---|---|---|
| ≤11 | 21.45 | 21.22 | 21.77 | 0.12 |
| 12 | 20.97 | 20.73 | 21.20 | 0.04 |
| 13 | 20.53 | 20.48 | 20.58 | 0.64 |
| 14 | 20.25 | 20.02 | 20.46 | 0.10 |
| ≥15 | 19.93 | 19.97 | 19.89 | 0.80 |
| Overall mean | 20.69[b] | 20.53[b] | 20.80[b] | 0.05 |

[a] *p* value for difference in mean BMI at age 18 between cases and controls by age at menarche; includes both *BRCA1* and *BRCA2* mutation carriers.
[b] $p < 0.0001$ by one-way ANOVA for between age comparisons in all, case and control subjects.

The effect of age at menarche on breast cancer risk was not mediated through childhood obesity. Childhood obesity has been shown to be a strong determinant of menarcheal age [12] and we found that increasing BMI at age 18 was associated with an earlier age of menarche. However, case subjects appeared to have a modestly lower BMI than control subjects, and the inclusion of BMI in the model actually strengthened the association

*Table 4B.* Comparison of mean BMI at age 18 according to age at menarche in *BRCA1* mutation carriers

| Age | All | Cases | Controls | *p* value[a] |
|---|---|---|---|---|
| ≤11 | 21.58 | 21.43 | 21.80 | 0.38 |
| 12 | 20.90 | 20.77 | 21.03 | 0.35 |
| 13 | 20.48 | 20.30 | 20.63 | 0.18 |
| 14 | 20.40 | 20.14 | 20.63 | 0.11 |
| ≥15 | 19.95 | 20.13 | 19.83 | 0.47 |
| Overall mean | 20.69[b] | 20.60[b] | 20.77[b] | 0.22 |

[a] *p* value for difference in mean BMI at age 18 between cases and controls by age at menarche; only includes *BRCA1* mutation carriers.
[b] $p < 0.0001$, $< 0.0001$ and 0.0001 by one-way ANOVA for between age comparisons in all *BRCA1*, case and control subjects, respectively.

between age at menarche and breast cancer risk. These results illustrate that age at menarche is a strong determinant of breast cancer risk, independent of the effects of BMI. The effect of weight, and of weight gain on breast cancer risk has been studied extensively in this cohort (Kotsopoulos *et al.* submitted).

Hamilton and Mack [13] examined the influence of age at puberty among pairs of female twins, one or both of whom had breast cancer. Among monozygotic twins, earlier puberty was associated with an earlier age of breast cancer diagnosis and was a strong predictor of diagnosis age when both twins were affected (OR = 5.4; 95% CI 2.0–14.5). This effect was not observed in the dizygotic twins (OR = 1.4; 95% CI 0.7–3.0). Because concordant monozygotic twins are believed to have a higher susceptibility to disease, this study suggests that age at menarche may play a greater role in genetically susceptible subgroups than in the general population. However, the *BRCA1* and *BRCA2* mutation status of these twins was not known.

In the general population, reproductive risk factors including early age at menarche, nulliparity, late age at first full-term pregnancy, and late age at menopause

*Table 4C.* Comparison of mean BMI at age 18 according to age at menarche in *BRCA2*mutation carriers

| Age | All | Cases | Controls | *p* value[a] |
|---|---|---|---|---|
| ≤11 | 21.13 | 20.71 | 21.69 | 0.38 |
| 12 | 21.12 | 20.64 | 21.50 | 0.35 |
| 13 | 20.69 | 20.99 | 20.44 | 0.18 |
| 14 | 19.67 | 19.57 | 19.77 | 0.11 |
| ≥15 | 19.86 | 19.66 | 20.11 | 0.47 |
| Overall mean[b] | 20.69 | 20.48 | 20.89 | 0.07 |

[a] *p* value for difference in mean BMI at age 18 between cases and controls by age at menarche; only includes *BRCA2* mutation carriers.
[b] $p < 0.0001$, 0.012 and 0.0009 by one-way ANOVA for between age comparisons in all *BRCA2*, case and control subjects, respectively.

are associated with an increased risk for breast cancer; whereas breastfeeding, higher parity and oophorectomy are protective (reviewed in [1, 2]). Henderson *et al.* [14] suggest that breast cancer risk is directly related to the cumulative number of regular ovulatory cycles and consequently to lifetime exposure of the breast to ovarian hormones. Colditz and Frazier [15, 16] (among others) have proposed models emphasizing the importance of early life exposures, especially prior to first childbirth, and the subsequent risk of breast cancer. Among *BRCA1* mutation carriers, breastfeeding is associated with a decreased risk of breast cancer [17]. The role of reproductive factors in women with a *BRCA2* mutation is less clear. Oophorectomy (removal of the ovaries) is protective in both subgroups [18–20].

The development of the female breast begins in embryonic life and continues throughout a women's lifetime [21]. During adolescence, mammary gland development includes the initiation of lobule formation resulting in the development of lobules type 1 within one to two years after the onset of menarche. The breasts of nulliparous women are predominantly composed of the undifferentiated type 1 lobules, whereas the breasts of parous women are predominantly composed of the more differentiated type 3 lobules. The higher proliferative index and concentration of steroid hormone receptors of the type 1 lobules makes this structure more susceptible to carcinogenic insult and thus type 1 lobules are considered to be the sites of origin of breast carcinomas [22]. Following pregnancy, the hormonally induced differentiation of the breast results in mammary cells which are less susceptible to carcinogens [22–24]. Thus, initiatives at delaying age at menarche may shorten this critical time period [25].

In a small study of 46 German *BRCA1* mutation carriers, menarche before the age of 14 was associated with a significantly earlier age of breast cancer onset compared with those with an age at menarche of ≥14 years old (*p*-log rank test = 0.04) [9]. Only one study has specifically evaluated whether there is a relationship between menarcheal age and the risk of breast cancer individuals with a *BRCA2* mutation [11]. Tryggvadottir *et al.* [11] reported no significant association with respect to age at menarche and breast cancer risk. Nonetheless, they did find that the effect of age at menarche on breast cancer risk was in the same direction for women with and without the *BRCA2* mutation, showing a decrease in risk with later menarcheal age. This effect was much stronger in the *BRCA2*-negative cases *versus* the *BRCA2*-positive cases. The results from the latter study cannot be extrapolated to all women with a *BRCA* mutation as the design was restricted to carriers of a

specific *BRCA2* founder mutation and was limited by the small sample size.

In the present study, a late age at menarche was associated with a significantly reduced risk of breast cancer in women with a *BRCA1* mutation, but not a *BRCA2* mutation. The protective effect in the former group may relate to increased expression of the *BRCA1* gene in breast tissues during periods of rapid proliferation and differentiation, such as embryogenesis, puberty, and pregnancy [26–28]. *BRCA1* expression is increased in rapidly proliferating murine mammary glands during puberty and pregnancy when levels of ovarian hormones are high [29–31]. *BRCA1* has also been shown to suppress estrogen-mediated breast cell proliferation, *in vitro* [32]. Furthermore, both *BRCA1* and *BRCA2* help maintain genomic integrity by protecting cells against oxidative stress by inducing expression of genes involved in antioxidant responses [33] and through participation in the cellular response to DNA damage, more specifically, the repair of double-stranded DNA breaks [34]. Russo *et al*. [35] have also reported that the developmental pattern of breast tissue from parous women with a family history of breast cancer or a *BRCA1* mutation was similar to that of nulliparous women suggesting a functional role of the *BRCA1* gene in the branching pattern of the breast during lobular development associated with pregnancy. Since the breasts of *BRCA1* mutation carriers may be composed predominantly of the undifferentiated, highly susceptible lobules type 1, during periods of active cellular proliferation in the breast (*i.e*. puberty) when both *BRCA1* expression and ovarian hormone production are normally elevated, women with a *BRCA1* mutation (one functional allele and subsequent decreased expression of *BRCA1*) may be especially susceptible to the carcinogenic effects of hormonal exposure. In individuals heterozygous for a deleterious *BRCA1* or *BRCA2* mutation, it seems likely that loss of the remaining normal copy of either of these genes would have much more impact if this event occurred in undeveloped breast tissue prior to puberty, rather than later on in more mature breast tissue. An early loss of *BRCA* gene function in cells of the undeveloped breast could conceivably give rise to the proliferation of sizable clones of cells without functional *BRCA1* or *BRCA2* during normal sexual maturation. This could potentially increase the number of cells highly vulnerable to genotoxic insult, which would markedly increase the likelihood of an event leading to genetic destabilization and uncontrolled cell growth.

The lack of a significant effect of age at menarche among *BRCA2* carriers may be attributed to the small sample size of *BRCA2* carriers or due to real physiologic differences. Evidence to date suggests that other reproductive factors (*e.g*. breastfeeding and parity) appear to play a less important role in the etiology of *BRCA2*-associated carcinogenesis. These findings will require confirmation in future studies.

The primary strength of our study is the large sample of known *BRCA* mutation carriers. We included 1311 matched pairs selected from a total of approximately 6133 documented mutation carriers, the largest study addressing the role of menarche on the risk of hereditary breast cancer. Our matching strategy and exclusion criteria resulted in case and control groups that were similar in most respects. A potential limitation of this study was the introduction of information bias with the use of self-reported data that may have resulted in non-differential misclassification. Although the women were required to recall early menstrual characteristics such as age at menarche, studies have shown a high correlation between recalled and original age at menarche ($r = 0.79$, $p < 0.001$) [36]. The potential of recall bias is minimal since there was no reason for the women to suspect such risk factors in the etiology of their disease.

We found that country of residence influenced age at menarche with women from North America experiencing menarche at a significantly earlier age (data not shown). In developed countries, secular trends are showing that age at menarche is declining and may be explained by a combination of factors that include improved nutrition, a sedentary lifestyle, the achievement of attained height at an earlier age and increases in adolescent height and weight (reviewed in [5]). In general, relatively tall [37, 38] and obese girls [38–40] undergo earlier menarche whereas physically active adolescents experience delayed menarche [41]. Lipworth [42] has proposed that a high fat diet in the years prior to adolescence may also accelerate age at menarche. We did not have information on childhood weight, height or physical activity and thus we were not able to consider these variables in our analyses.

Although our study was limited to women with a genetic predisposition to breast cancer, our findings are in agreement with the belief that an earlier age at menarche is a risk factor for breast cancer development in general. Girls who undergo early sexual maturation, as determined by menarcheal age, tend to be obese as adults [43–45]. The risk of adult obesity is also greater for those who were obese as children [46], hence incentives directed at delaying age at menarche may also help decrease the risk of adult obesity and the resultant metabolic consequences such as hyperinsulinemia, glucose intolerance, and type 2 diabetes [47]. The prevalence of childhood obesity is increasing [48] and nutritional status as a child is

positively related to both childhood height and weight, two important determinants of menarche [5]. Studies have clearly shown that dietary energy intake [49], height [50], weight [40] and various lifestyle factors such as reduced physical activity [16, 51] throughout childhood years may trigger early onset puberty. Our findings point towards the importance of environmental or lifestyle factors as possible predictors of menarcheal age and represent potential modifiers to help delay menarcheal age and perhaps the risk of breast cancer.

## Acknowledgements

Supported by a grant from the Canadian Breast Cancer Research Alliance. Susan L. Neuhausen is supported by a grant from the National Institutes of Health (NIH CA74415). We thank B. Rosen, G. Rennert, C. Eng, J. McLennan, W. McKinnon, M. Wood, S. Merajver, D. Fishman, D. Provencher, J. Weitzel, G. Evans, P. Moller, W.S. Meschino, E. Lemire and T. Wagner for submission of data on their patients.

## References

1. Kelsey JL, Gammon MD, John EM (1993) Reproductive factors and breast cancer. *Epidemiol Rev* **15** (1): 36–47.
2. Key TJ, Verkasalo PK, Banks E (2001) Epidemiology of breast cancer. *Lancet Oncol* **2** (3): 133–140.
3. Kuller LH (1995) The etiology of breast cancer – from epidemiology to prevention. *Public Health Rev* **23** (2): 157–213.
4. Okasha M, *et al.* (2001) Age at menarche: secular trends and association with adult anthropometric measures. *Ann Hum Biol* **28** (1): 68–78.
5. Petridou E, *et al.* (1996) Determinants of age at menarche as early life predictors of breast cancer risk. *Int J Cancer* **68** (2): 193–198.
6. Parazzini F, *et al.* (1992) Menstrual and reproductive factors and breast cancer in women with family history of the disease. *Int J Cancer* **51** (5): 677–681.
7. Magnusson C, *et al.* (1998) Association of family history and other risk factors with breast cancer risk (Sweden). *Cancer Causes Control* **9** (3): 259–267.
8. Olsson H, *et al.* (1985) Risk factors of breast cancer in relation to family history of breast cancer in Southern Sweden In: Muller H, Weber W, eds. *Familial Cancer,* Basel: Karger, pp. 34–35. .
9. Chang-Claude J, *et al.* (1997) Modifying effect of reproductive risk factors on the age at onset of breast cancer for German BRCA1 mutation carriers. *J Cancer Res Clin Oncol* **123** (5): 272–279.
10. Jernstrom HC, *et al.* (1999) Reproductive factors in hereditary breast cancer. *Breast Cancer Res Treat* **58** (3): 295–301.
11. Tryggvadottir L, *et al.* (2003) *BRCA2* mutation carriers, reproductive factors and breast cancer risk. *Breast Cancer Res.* **5** (5): R121–R128.
12. Freedman DS, *et al.* (2003) The relation of menarcheal age to obesity in childhood and adulthood: the Bogalusa heart study. *BMC Pediatr* **3** (1): 3.
13. Hamilton AS, Mack TM (2003) Puberty and genetic susceptibility to breast cancer in a case–control study in twins. *N Engl J Med* **348** (23): 2313–2322.
14. Henderson BE, *et al.* (1985) Do regular ovulatory cycles increase breast cancer risk?. *Cancer* **56** (5): 1206–1208.
15. Cole P, MacMahon B (1969) Oestrogen fractions during early reproductive life in the aetiology of breast cancer. *Lancet* **1** (7595): 604–606.
16. Colditz GA, Frazier AL (1995) Models of breast cancer show that risk is set by events of early life: prevention efforts must shift focus. *Cancer Epidemiol Biomarkers Prev* **4** (5): 567–571.
17. Jernstrom H, *et al.* (2004) *Breast-Feeding and the Risk of Cancer in BRCA1 and BRCA2 Carriers, JNCI.*
18. Narod SA, *et al.* (1995) Risk modifiers in carriers of *BRCA1* mutations. *Int J Cancer* **64** (6): 394–398.
19. Rebbeck TR, *et al.* (1999) Breast cancer risk after bilateral prophylactic oophorectomy in *BRCA1* mutation carriers. *J Natl Cancer Inst* **91** (17): 1475–1479.
20. Eisen A (2000) Reduction in breast cancer risk following bilateral prophylactic oophorectomy in *BRCA1* and *BRCA2* mutation carriers. *Am J Hum Genet* **67** (58).
21. Russo J, Russo IH (2004) Development of the human breast. *Maturitas* **49** (1): 2–15.
22. Russo J, Rivera R, Russo IH (1992) Influence of age and parity on the development of the human breast. *Breast Cancer Res Treat* **23** (3): 211–218.
23. Russo J, Russo IH (1994) Toward a physiological approach to breast cancer prevention. *Cancer Epidemiol Biomarkers Prev* **3** (4): 353–364.
24. Russo J, Russo IH (1999) Cellular basis of breast cancer susceptibility. *Oncol Res* **11** (4): 169–178.
25. Russo J, Tay LK, Russo IH (1982) Differentiation of the mammary gland and susceptibility to carcinogenesis. *Breast Cancer Res Treat* **2** (1): 5–73.
26. Hakem R, *et al.* (1996) The tumor suppressor gene *Brca1* is required for embryonic cellular proliferation in the mouse. *Cell* **85** (7): 1009–1023.
27. Marquis ST, *et al.* (1995) The developmental pattern of *Brca1* expression implies a role in differentiation of the breast and other tissues. *Nat Genet* **11** (1): 17–26.
28. Vaughn JP, *et al.* (1996) *BRCA1* expression is induced before DNA synthesis in both normal and tumor-derived breast cells. *Cell Growth Differ* **7** (6): 711–715.
29. Lane TF, *et al.* (1995) Expression of *Brca1* is associated with terminal differentiation of ectodermally and mesodermally derived tissues in mice. *Genes Dev* **9** (21): 2712–2722.
30. Rajan JV, *et al.* (1997) Developmental expression of *Brca2* colocalizes with *Brca1* and is associated with proliferation and differentiation in multiple tissues. *Dev Biol* **184** (2): 385–401.
31. Kubista M, *et al.* (2002) *Brca1* regulates *in vitro* differentiation of mammary epithelial cells. *Oncogene* **21** (31): 4747–4756.
32. Fan S, *et al.* (1999) *BRCA1* inhibition of estrogen receptor signaling in transfected cells. *Science* **284** (5418): 1354–1356.
33. Bae I, *et al.* (2004) *BRCA1* induces antioxidant gene expression and resistance to oxidative stress. *Cancer Res* **64** (21): 7893–7909.
34. Scott RJ (2004) DNA double strand break repair and its association with inherited predispositions to breast cancer. *Hereditary Cancer Clin Practice* **2** (1): 37–43.

35. Russo J, Lynch H, Russo IH (2001) Mammary gland architecture as a determining factor in the susceptibility of the human breast to cancer. *Breast J* **7** (5): 278–291.
36. Must A, *et al.* (2002) Recall of early menstrual history and menarcheal body size: after 30 years, how well do women remember. *Am J Epidemiol* **155** (7): 672–679.
37. Ellison PT (1982) Skeletal growth, fatness and menarcheal age: a comparison of two hypotheses. *Hum Biol* **54** (2): 269–281.
38. Moisan J, Meyer F, Gingras S (1990) A nested case–control study of the correlates of early menarche. *Am J Epidemiol* **132** (5): 953–961.
39. Frisch RE, Revelle R (1970) Height and weight at menarche and a hypothesis of critical body weights and adolescent events. *Science* **169** (943): 397–399.
40. Kaplowitz PB, *et al.* (2001) Earlier onset of puberty in girls: relation to increased body mass index and race. *Pediatrics* **108** (2): 347–353.
41. Merzenich H, Boeing H, Wahrendorf J (1993) Dietary fat and sports activity as determinants for age at menarche. *Am J Epidemiol* **138** (4): 217–224.
42. Lipworth L (1995) Epidemiology of breast cancer. *Eur J Cancer Prev* **4** (1): 7–30.
43. Sharma K, Talwar I, Sharma N (1988) Age at menarche in relation to adult body size and physique. *Ann Hum Biol* **15** (6): 431–434.
44. van Lenthe FJ, Kemper CG, Mechelen Wvan (1996) Rapid maturation in adolescence results in greater obesity in adulthood: the Amsterdam Growth and Health Study. *Am J Clin Nutr* **64** (1): 18–24.
45. Laitinen J, Power C, Jarvelin MR (2001) Family social class, maternal body mass index, childhood body mass index, and age at menarche as predictors of adult obesity. *Am J Clin Nutr* **74** (3): 287–294.
46. Serdula MK, *et al.* (1993) Do obese children become obese adults? A review of the literature. *Prev Med* **22** (2): 167–177.
47. James PT, Rigby N, Leach R (2004) The obesity epidemic, metabolic syndrome and future prevention strategies. *Eur J Cardiovasc Prev Rehabil* **11** (1): 3–8.
48. Ogden CL, *et al.* (2002) Prevalence and trends in overweight among US children and adolescents, 1999–2000. *Jama* **288** (14): 1728–1732.
49. Meyer F, *et al.* (1990) Dietary and physical determinants of menarche. *Epidemiology* **1** (5): 377–381.
50. Frisch RE, Revelle R (1971) Height and weight at menarche and a hypothesis of menarche. *Arch Dis Child* **46** (249): 695–701.
51. Baxter-Jones AD, *et al.* (1994) Menarche in intensively trained gymnasts, swimmers and tennis players. *Ann Hum Biol* **21** (5): 407–415.