Research Article

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# Breast Cancer Risk Associated with Estrogen Exposure and Truncating Mutation Location in *BRCA1/2* Carriers

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## Abstract

**Background:** Mutations in *BRCA1/2* confer a high risk of breast cancer, but literature values of this risk vary. A genotype–phenotype correlation has been found in both genes, and the effect of reproductive factors differs according to mutation location. Therefore, we hypothesize that such a variation may exist for other factors related to estrogen exposure.

**Methods:** We used a weighted Cox regression model to assess variation in breast cancer risk with these factors using location of mutation in homogeneous breast cancer risk region of *BRCA1/2* in the GENEPSO study.

**Results:** We found that late age at menarche reduced breast cancer risk by 31% and that among *BRCA1* carriers, a long or a short menstrual cycle increased risk (by 65% and 73%, respectively). Among premenopausal women, overweight was associated with a 45% decrease in risk whereas underweight was associated with an increased risk (HR, 2.40). A natural menopause, mainly after age 50, was associated with a high breast cancer risk (HR, 2.46), and a significant interaction between menopause status and the location of mutations was found leading up to 10% variation in absolute risk according to the age at menopause.

**Conclusions:** As observed in the general population, a late menarche, a long or a short menstrual cycle, over- or underweight, and being postmenopausal were associated with breast cancer risk in *BRCA1/2* carriers. The association with the menopause was observed only when the mutation was located in the "high-risk" zones.

**Impact:** Taking into account modifier factors, location of mutation might be important for the clinical management of *BRCA1/2* mutation carriers. *Cancer Epidemiol Biomarkers Prev; 24(4); 698–707. ©2015 AACR.*



## Introduction

Estrogens are known to induce mammary tumors in animals. Several epidemiologic studies support the hypothesis that estrogens play an important role in the development of breast cancer. Indeed, breast cancer risk increases with exposure to ovarian hormones, and it is now well established that early menarche and late menopause are risk factors in the general population, whereas premenopausal oophorectomy decreases the risk (1).

Among *BRCA1/2* mutation carriers, the few studies that have assessed the risk of breast cancer associated with age at menarche

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**Note:** Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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(e.g., ref. 2–6), menopausal status (2), oral contraceptive (OC; refs. 7–11), hormonal replacement therapy (HRT; e.g. ref. 12, 13), and body mass index (BMI; e.g., refs. 14, 15) have shown mainly inconsistent results, except for oophorectomy (e.g., refs. 16, 17). In their meta-analysis to study potential modifiers of breast cancer risk in *BRCA1/2* mutation carriers, Friebel and colleagues (18) emphasized the limitations in the existing literature, which could explain the inconsistency in the results. Indeed, the studies published so far were either case–control studies, retrospective or prospective cohorts. Many of them involved small sample sizes and retrospective measurement of the risk factors leading to potential recall bias. They had different ascertainment of carriers, time-dependent variables analyzed either as fixed or as time dependent etc. Friebel and colleagues (18) could not identify incontrovertible associations and in addition to the limitations mentioned above, they noted that any of the studies accounted for breast tumor characteristics or for mutational heterogeneity that also could be source of inconsistency. Indeed, in both *BRCA1* and *BRCA2*, a genotype–phenotype correlation has been found (e.g., refs. 19, 20). In a previous publication, we confirmed (21) the existence of a central low breast cancer–risk region in *BRCA1* and *BRCA2*, described a new high-risk region in *BRCA2* and studied the effect of factors related to pregnancy for these homogeneous regions. Our results suggested a variation in breast cancer risk associated with parity according to the location of the mutation in *BRCA1*, that is, a loss of the protective effect of parity when the mutation fell in the N-terminal and the C-terminal regions. This observation was consistent with the hypothesis of the *BRCA1* cellular antiproliferative effect via inhibition of the transcriptional activity of estrogen receptor α through protein–protein interaction in these regions (22–25). Therefore, we hypothesize that variation may also exist for the effect of factors related to exposure to estrogens, either endogens or exogens, like age at menarche, menopausal status, BMI, OC, and HRT use.

## Materials and Methods

### Data

The GENEPSO study was initiated in 2000 to estimate the risk of breast, ovarian, and other cancers in *BRCA1/2* mutation carriers, and to assess potential risk-modifying factors, either lifestyle or genetic. Subjects were from the family cancer clinics of the Genetic and Cancer Group of Unicancer. Any woman carrying a pathogenic mutation in the *BRCA1* or *BRCA2* gene was eligible, including those diagnosed with cancer and those currently unaffected. They had to be at least 18 years old, mentally capable of giving informed consent to study participation, and had been counseled about their mutation status. The research protocol was approved by the relevant ethics committees, and all participants provided written informed consent.

The study population was based on the women enrolled in the GENEPSO study from 2000. A total of 1,337 women were recruited, 863 were *BRCA1* mutation carriers, and 474 were *BRCA2* mutation carriers. Around 563 women had been diagnosed with breast cancer at the time of their interview, but only 499 of them were considered as affected in this analysis after censoring. The remaining 838 women were censored at age at diagnosis of ovarian cancer ($N = 89$), at diagnosis of another cancer ($N = 16$), at prophylactic bilateral mastectomy ($N = 11$), or at interview ($N = 722$). To assess variation in breast cancer risk according to mutation position, a sample with one subject per family (i.e., 990 women) was randomly selected to avoid overmatching on a specific mutation. The random choice of one woman per family affected 30% of the families analyzed.

A detailed and standardized questionnaire on reproductive and lifestyle factors (e.g., weight at interview and before diagnosis for affected women, height, ages at menarche and menopause, menstrual cycle duration, OC, and HRT use. . .) was administered by mail.

### Mutation screening

The mutation screening strategy was similar for all the clinics, that is, the youngest living affected family member was tested first and, if a *BRCA1/2* mutation was found, affected and unaffected family members were offered testing.

The full coding sequences and the exon–intron junctions of the *BRCA1* and *BRCA2* genes were screened for variants, based on prescreening [denaturing gradient gel electrophoresis, single-strand conformation polymorphism, protein truncation assay, denaturing high-performance liquid chromatography, high-resolution melting (HRM), or enhanced mismatch mutation analysis (EMMA)] and sequencing. Several large rearrangements were identified by large cDNA sequencing, multiplex ligation-dependent probe amplification, quantitative multiplex PCR of short fragments, quantitative PCR (qPCR), quantitative PCR high-resolution melting (qPCR HRM), EMMA, bar code screening, or dedicated array comparative genomic hybridization. Mutation description was provided by each French laboratory, coded, and standardized according to the international nomenclature.

### Statistical analysis

The data were analyzed using a modified Cox proportional hazards regression model to take into account that women included in this study were taken from high-risk families qualifying for genetic testing (26). The disease status may, therefore, have affected the likelihood of ascertainment and selection leading to an oversampling of affected women. To correct for this potential bias, the Cox regression analyses were performed using the weighted regression approach described by Antoniou and colleagues (26). Individuals were weighted such that the observed breast cancer incidence rates in the study sample were consistent with established breast cancer risk estimates for *BRCA1* and *BRCA2* carriers (27). The affected mutation carriers were underweighted (weights <1) and the unaffected mutation carriers were overweighted (weights >1). The weights were applied to all person-years of each subject in the modified Cox model.

Subjects were followed up from birth and censored at the date of diagnosis, for women who were affected by any cancer, or the date of prophylactic bilateral mastectomy or interview, for unaffected women. Menopausal status, OC, and HRT were analyzed as a time-dependent covariate, BMI and age at menarche as fixed. Weight was asked at the time of interview and before the diagnosis for women who were affected by any cancer. When information on the weight before the diagnosis was missing, information on current weight was used.

All analyses were stratified by period of birth (before 1940, 1940–1949, 1950–1959, 1960 or later). Because of potential confounder effects, analyses were adjusted for menopausal status

(yes/no), OC use (ever/never), parity (0, 1, 2, ≤3), HRT use (ever/never), and gene (*BRCA1* or *BRCA2*) accordingly.

Deleterious mutations were either truncating, missense, in-frame deletions, splice mutations, or partial/entire gene deletions. Because truncating mutations account for 90% of the mutations in our dataset and represent a homogeneous type of mutations, we performed a previous analysis to assess variation in breast cancer risk according to location of these mutations. This analysis led to homogeneous regions in breast cancer risk (21). Therefore; to assess the variation of breast cancer risk associated with covariates by location of truncating mutations in *BRCA1/2*, we used these regions defined in our previous publication (21) and illustrated in Supplementary Data. We considered two groups of mutations in *BRCA1*, those located in LR1 (for "low-risk region in *BRCA1*": codons 374 to 1161) and those located outside LR1. In *BRCA2*, we considered three groups of mutations in *BRCA2*, those located in LR2 (for "low-risk region in *BRCA2*": codons 957 to 1827), those located in HR2 (for "high-risk region in *BRCA2*": codons 2546 to 2968), and those located outside LR2 and HR2 (Supplementary Fig. S1).

All statistical analyses were two-sided and were performed using the STATA statistical package (version 12; Stata Corporation).

## Results

Characteristics of the whole cohort and of one-woman-per-family cohort are listed in Table 1. In the whole cohort, the average age at censoring for the 838 participants without breast cancer was 40.0 years (SD = 0.4), which is similar to the age at diagnosis of the women with breast cancer (41.0 years, SD = 0.4), although the age at interview was substantially greater for the patients with breast cancer, reflecting the pattern of genetic testing among participants. Sampling of one woman per family did not change any characteristic distribution or the average of age at censure (39.8 and 40.4 years, respectively, for women without and with breast cancer). There were a total of 39,666 person-years of observation.

Overall, women who reported having menarche at age 12 or older had a significant 31% decrease in the risk of breast cancer compared with women who had menarche before age 12, but this association only remained among *BRCA1* mutation carriers (Table 2). *BRCA1* mutation carriers with short (between 20 and 24 days) or long menstrual cycles (> 31 days) had a 73% increase in the risk of breast cancer compared with those having menstrual cycle duration between 24 and 31 days. Long cycles were associated with nonsignificant 65% and 69% increases in breast cancer risk when compared with menstrual cycle duration of between 24 and 31 days, for *BRCA1* and *BRCA2* carriers respectively.

Table 2 shows that current OC use was associated with a nonsignificant reduction in risk of breast cancer, and past OC use was associated with a nonsignificant 21% increase in risk. Past OC use had a stronger and significant effect when the time since last use was between 2 and 5 years when compared with current users, whatever the gene (HR, 1.65 and 3.40 for *BRCA1* and *BRCA2*, respectively). There was no significant association with the duration of use or with the age at first use.

Postmenopause seems to be associated with a higher breast cancer risk among *BRCA1* mutation carriers (post- vs. premenopause: HR, 1.63; Table 3). The point estimate associated with menopause among *BRCA2* mutation carriers was 0.54. This decrease in risk following the menopause does not seem to be explained by the protective effect of an oophorectomy, because the natural menopause was still associated with breast cancer, with similar estimates. Among *BRCA1* mutation carriers, the increase in breast cancer risk remained only for a natural menopause [HR, 2.14; 95% confidence interval (CI), 1.16–3.93, surgical menopause vs. premenopause: HR, 0.99; $P = 0.99$). Because very few women had a surgical menopause (289 person-years), the effect of age at menopause was studied only for a natural menopause. For *BRCA1* mutation carriers, we observed a 4.84-fold increase in risk of breast cancer associated with a natural menopause after age 50 compared with premenopausal women. The time since a natural menopause had no additional effect. Among postmenopausal women, current users of HRT were not at a significantly increased risk of developing breast cancer when compared with never users (Table 3). A significant association was found only when the duration of HRT use was more than 5 years and among *BRCA1* mutation carriers. We could not assess the effect of HRT cessation and time since last use, as too few women had stopped HRT.

Whatever the gene mutated, overweight (BMI > 25) was associated with a 39% decrease in breast cancer risk when compared with women with a BMI defined as normal (between 18.5 and 25), whereas underweight (BMI <18.5) was associated with a 109% increase in breast cancer risk (Table 4). Sensitivity analysis was performed, excluding the 149 women affected by cancer with missing information on weight before diagnosis and results were similar (BMI > 25: HR, 0.52; 95% CI, 0.35–0.77; $P = 0.001$; BMI <18.5: HR, 1.91; 95% CI, 1.09–3.33; $P = 0.024$).

These associations were significant only among premenopausal women, but follow-up of postmenopausal women was too short to assess the effect of BMI after the menopause. Height had no significant effect on the risk of breast cancer, although the point estimate increases as height increases. A significant trend was found for the women carrying a mutation in *BRCA2* ($P_{trend} = 0.021$)

For both genes, there was no interaction between the location of the mutation and all studied factors (data not shown), except for menopausal status (Tables 5 and 6) . Among *BRCA1* mutation carriers, menopause was significantly associated with an increased risk of breast cancer only among women with a mutation outside LR1 (HR, 2.15) and for women with a natural menopause (HR, 2.97), particularly with a menopause after age 50 (HR, 5.31). Among women with a mutation inside LR1, very few women were postmenopausal, though a natural menopause was associated with a 63% decrease in breast cancer risk ($P_{interaction} = 0.04$). For *BRCA2*, a significantly increased risk of breast cancer was observed among women with a mutation in HR2 (HR, 4.01) and a 19% decrease in risk was observed for women with a mutation outside LR2 and HR2 ($P_{interaction} = 0.02$; Tables 5 and 6). Again, these associations were almost explained by the effect of a natural menopause. The effect of menopause among women with a mutation in LR2 could not be assessed because no cases were postmenopausal. To assess how many these results could be affected by the random choice of one woman per family, we resampled the subjects and did the analyses on 20 samples. These analyses showed that this design has very little impact on results. For the menopause analyses, for example, the distribution of HR estimate for *BRCA1* mutation carriers with a mutation outside LR1 varied from 1.58 to 2.77 (65% significant) and for women with a

**Table 1.** Characteristics of the cohort study of *BRCA1/2* mutation carriers

| | Whole cohort | | | One woman per family sample cohort | | |
|---|---|---|---|---|---|---|
| | **Without breast cancer (*n* = 838)** | **With breast cancer (*n* = 499)** | **All women (*n* = 1,337)** | **Without breast cancer (*n* = 611)** | **With breast cancer (*n* = 379)** | **All women (*n* = 990)** |
| Characteristics | *N* (%) | *N* (%) | *N* (%) | *N* (%) | *N* (%) | *N* (%) |
| Mutation | | | | | | |
| *BRCA1* | 531 (63) | 332 (67) | 863 (65) | 395 (65) | 240 (63) | 635 (64) |
| *BRCA2* | 307 (37) | 167 (33) | 474 (35) | 216 (35) | 139 (37) | 355 (36) |
| Age at interview, y | | | | | | |
| Mean | 41.0 | 49.4 | 44.1 | 40.7 | 48.6 | 43.7 |
| SD | 0.4 | 0.5 | 0.3 | 0.5 | 0.5 | 0.4 |
| IC | 40.1–41.9 | 48.4–50.3 | 43.4–44.8 | 39.7–41.7 | 47.5–49.7 | 43.0–44.5 |
| Minimum and maximum | 18–99 | 27–87 | 18–99 | 18–99 | 27–83 | 18–99 |
| Age at diagnosis/censoring, y | | | | | | |
| Mean | 40.0 | 41.0 | 40.4 | 40.1 | 40.4 | 39.8 |
| SD | 0.4 | 0.4 | 0.3 | 0.3 | 0.5 | 0.5 |
| IC | 39.2–40.8 | 40.2–41.7 | 39.8–41.0 | 39.4–40.7 | 39.6–41.3 | 38.9–40.7 |
| Minimum and maximum | 18–99 | 23–77 | 18–99 | | | |
| <30 | 162 (19) | 34 (7) | 196 (15) | 142 (23) | 29 (8) | 113 (11) |
| 30–39 | 282 (34) | 205 (41) | 487 (36) | 371 (61) | 159 (42) | 212 (21) |
| 40–49 | 227 (27) | 176 (35) | 403 (30) | 306 (50) | 133 (35) | 173 (17) |
| 50–59 | 113 (13) | 67 (13) | 180 (13) | 126 (21) | 47 (12) | 79 (8) |
| ≥60 | 54 (6) | 17 (3) | 71 (5) | 45 (7) | 11 (3) | 34 (3) |
| Time between interview and diagnosis | | | | | | |
| Mean | | 8.4 | | | 8.2 | |
| SD | | 7.0 | | | 6.6 | |
| IC | | 7.8–9.00 | | | 7.50–8.84 | |
| Minimum and maximum | | 0–39.3 | | | 0–34 | |
| Year of birth | | | | | | |
| <1950 | 153 (18) | 201 (40) | 354 (26) | 237 (39) | 139 (37) | 98 (10) |
| 1950–1960 | 159 (19) | 165 (33) | 324 (24) | 248 (41) | 128 (34) | 120 (12) |
| 1960–1970 | 232 (28) | 119 (24) | 351 (26) | 282 (46) | 99 (26) | 183 (18) |
| ≥1970 | 294 (35) | 14 (3) | 308 (23) | 223 (36) | 13 (3) | 210 (21) |
| Age at menarche, y | | | | | | |
| <12 | 129 (15) | 98 (20) | 227 (17) | 96 (16) | 74 (20) | 170 (17) |
| [12–15] | 592 (71) | 317 (64) | 909 (68) | 435 (71) | 239 (63) | 674 (68) |
| >15 | 110 (13) | 84 (17) | 194 (15) | 77 (13) | 66 (17) | 143 (14) |
| Never | 4 (0) | 0 (0) | 4 (0) | 2 (0) | 0 (0) | 2 (0) |
| Missing | 2 (0) | 0 (0) | 2 (0) | 1 (0) | 0 (0) | 1 (0) |
| Number of full-term pregnancies | | | | | | |
| 0 | 225 (27) | 68 (14) | 293 (22) | 159 (26) | 58 (15) | 217 (22) |
| 1 | 142 (17) | 108 (22) | 250 (19) | 106 (17) | 90 (24) | 196 (20) |
| 2 | 270 (32) | 181 (36) | 451 (34) | 207 (34) | 138 (36) | 345 (35) |
| ≥3 | 201 (24) | 141 (28) | 342 (26) | 139 (23) | 92 (24) | 231 (23) |
| Missing | 0 (0) | 1 (0) | 1 (0) | 0 (0) | 1 (0) | 1 (0) |
| Oral contraceptive use during more than 6 months | | | | | | |
| Never | 159 (19) | 130 (26) | 289 (22) | 108 (18) | 93 (25) | 201 (20) |
| Ever | 635 (76) | 337 (68) | 972 (73) | 469 (77) | 259 (68) | 728 (74) |
| Missing | 44 (5) | 32 (6) | 76 (6) | 34 (6) | 27 (7) | 61 (6) |
| Menopausal status | | | | | | |
| Premenopausal | 664 (79) | 404 (81) | 1,068 (80) | 483 (79) | 312 (82) | 795 (80) |
| Postmenopausal | 154 (18) | 86 (17) | 240 (18) | 111 (18) | 60 (16) | 171 (17) |
| Missing | 20 (2) | 9 (2) | 29 (2) | 17 (3) | 7 (2) | 24 (2) |
| HRT use | | | | | | |
| Never | 93 (11) | 54 (11) | 147 (11) | 70 (11) | 40 (11) | 110 (11) |
| Ever | 60 (7) | 31 (6) | 91 (7) | 40 (7) | 19 (5) | 59 (6) |
| Premenopausal | 662 (79) | 404 (81) | 1,066 (80) | 481 (79) | 312 (82) | 793 (80) |
| Missing | 23 (3) | 10 (2) | 33 (2) | 20 (3) | 8 (2) | 28 (3) |
| BMI | | | | | | |
| <18.5 | 579 (69) | 359 (72) | 938 (70) | 429 (70) | 275 (73) | 704 (71) |
| [18.5–25] | 210 (25) | 106 (21) | 316 (24) | 147 (24) | 75 (20) | 222 (22) |
| >25 | 46 (5) | 33 (7) | 79 (6) | 32 (5) | 29 (8) | 61 (6) |
| Missing | 3 (0) | 1 (0) | 4 (0) | 3 (0) | 0 (0) | 3 (0) |

natural menopause from 1.95 to 4.49 (100% significant). Among women with a mutation inside LR1, a natural menopause was associated with a decrease in breast cancer risk varying from 0.28 to 0.81 (no significant). For *BRCA2* mutation carriers with a mutation in HR2, the distribution of HR estimate varied from 3.27 to 9.64 (90% significant) and from 0.57 to 1.18 for women with a mutation outside LR2 and HR2 (no significant). Forty-five percent of the interaction tests between a natural menopause and mutation position were significant for *BRCA1* and 95% for *BRCA2*.

**Table 2.** Risk of breast cancer associated with age at menarche, duration of menstrual cycle, and oral contraceptive use

| | One woman per family cohort: 39,666 person-years of follow-up | | | | *BRCA1* mutation carriers: 25,045 person-years of follow-up | | | | *BRCA2* mutation carriers: 14,621 person-years of follow-up | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Person-years[a] | Number of cases[a] | HR[b] (95% CI) | *P* | Person-years[a] | Number of cases[a] | HR[c] (95% CI) | *P* | Person-years[a] | Number of cases[a] | HR[c] (95% CI) | *P* |
| Age at menarche (y) | | | | | | | | | | | | |
| <12 | 6,522 | 74 | 1.00 | | 4,253 | 54 | 1.00 | | 2,269 | 20 | 1.00 | |
| 12–15 | 27,153 | 239 | 0.66 (0.46–0.93) | 0.02 | 17,003 | 145 | 0.60 (0.40–0.89) | 0.01 | 10,150 | 94 | 1.04 (0.52–2.09) | 0.91 |
| >15 | 5,887 | 66 | 0.84 (0.53–1.34) | 0.47 | 3,685 | 41 | 0.74 (0.43–1.26) | 0.27 | 2,202 | 25 | 1.68 (0.71–3.95) | 0.24 |
| Never | 76 | 0 | | | 76 | 0 | | | 0 | 0 | | |
| Age at menarche (y) | | | | | | | | | | | | |
| <12 | 6,522 | 74 | 1.00 | | 4,253 | 54 | 1.00 | | 2,269 | 20 | 1.00 | |
| ≥12 | 33,040 | 305 | 0.69 (0.49–0.97) | 0.03 | 20,688 | 186 | 0.62 (0.42–0.92) | 0.02 | 12,352 | 119 | 1.13 (0.57–2.23) | 0.73 |
| Never | 76 | 0 | | | 76 | 0 | | | 0 | 0 | | |
| Menstrual cycle duration | | | | | | | | | | | | |
| 24–31 days | 27,076 | 241 | 1.00 | | 16,862 | 148 | 1.00 | | 10,214 | 93 | 1.00 | |
| 20–24 days | 4,009 | 46 | 1.48 (1.01–2.19) | 0.05 | 2,429 | 31 | 1.73 (1.12–2.68) | 0.01 | 1,580 | 15 | 0.93 (0.40–2.18) | 0.87 |
| >31 days | 1,898 | 21 | 1.65 (0.95–2.86) | 0.08 | 1,190 | 13 | 1.65 (0.84–3.24) | 0.15 | 708 | 8 | 1.69 (0.63–4.56) | 0.30 |
| Oral contraceptive use | | | | | | | | | | | | |
| Never | 23,972 | 93 | 1.00 | | 15,260 | 59 | 1.00 | | 8,712 | 34 | 1.00 | |
| Current | 8,088 | 65 | 0.77 (0.51–1.16) | 0.21 | 5,353 | 50 | 0.80 (0.50–1.28) | 0.35 | 2,735 | 15 | 0.61 (0.27–1.39) | 0.24 |
| Past | 5,775 | 194 | 1.21 (0.86–1.71) | 0.27 | 3,415 | 118 | 1.08 (0.72–1.62) | 0.72 | 2,360 | 76 | 1.71 (0.90–3.23) | 0.10 |
| Duration of oral contraceptive use (y) | | | | | | | | | | | | |
| Never | 23,972 | 93 | 1.00 | | 15 260 | 59 | 1.00 | | 8,712 | 34 | 1.00 | |
| ≤5 | 6,333 | 78 | 1.21 (0.80–1.82) | 0.36 | 3,827 | 47 | 1.19 (0.74–1.92) | 0.48 | 2,506 | 31 | 1.37 (0.64–2.91) | 0.42 |
| >5 | 7,464 | 180 | 1.03 (0.72–1.47) | 0.86 | 4,894 | 120 | 0.91 (0.60–1.37) | 0.65 | 2,570 | 60 | 1.51 (0.77–2.95) | 0.23 |
| Time since last oral contraceptive use (y) | | | | | | | | | | | | |
| <1 | 8,088 | 65 | 1.00 | | 5,353 | 50 | 1.00 | | 2,735 | 15 | 1.00 | |
| 2–5 | 2,401 | 66 | 1.87 (1.27–2.75) | $<10^{-3}$ | 1,476 | 44 | 1.65 (1.05–2.60) | 0.03 | 925 | 22 | 3.40 (1.65–7.00) | $<10^{-3}$ |
| 6–10 | 1,473 | 45 | 1.53 (0.99–2.37) | 0.06 | 865 | 34 | 1.46 (0.88–2.40) | 0.14 | 608 | 11 | 1.60 (0.69–3.74) | 0.27 |
| >10 | 1,897 | 83 | 1.41 (0.92–2.17) | 0.12 | 1,070 | 40 | 1.06 (0.63–1.79) | 0.82 | 827 | 43 | 3.07 (1.46–6.45) | $<10^{-3}$ |
| Age at first oral contraceptive use (y) | | | | | | | | | | | | |
| Never | 23,972 | 93 | 1.00 | | 15,260 | 59 | 1.00 | | 8,712 | 34 | 1.00 | |
| <20 | 8,931 | 149 | 1.27 (0.84–1.93) | 0.25 | 5,656 | 100 | 1.23 (0.74–2.02) | 0.42 | 3,275 | 49 | 1.43 (0.69–2.96) | 0.34 |
| 20–25 | 3,306 | 68 | 1.00 (0.65–1.55) | 0.99 | 2,150 | 42 | 0.87 (0.52–1.46) | 0.60 | 1,156 | 26 | 1.44 (0.63–3.29) | 0.38 |
| >25 | 1,627 | 42 | 1.01 (0.63–1.62) | 0.96 | 962 | 26 | 0.95 (0.54–1.66) | 0.85 | 665 | 16 | 1.48 (0.65–3.33) | 0.35 |

[a]Not including missing data.

[b]For age at menarche and menstrual cycle duration: adjusted for parity (0, 1, 2, ≥3), menopausal status (premenopause and postmenopause), oral contraceptive use (yes or no), and mutated gene (*BRCA1* or *BRCA2*). For oral contraceptive use: adjusted for parity (0, 1, 2, ≥3), menopausal status (premenopause and postmenopause), and mutated gene (*BRCA1* or *BRCA2*).

[c]For age at menarche and menstrual cycle duration: adjusted for parity (0, 1, 2, ≥3), menopausal status (premenopause and postmenopause), and oral contraceptive use (yes or no). For oral contraceptive use: adjusted for parity (0, 1, 2, ≥3) and menopausal status (premenopause and postmenopause).

Figure 1 using breast cancer incidence rates estimated by Mavaddat and colleagues (28), shows that *BRCA1* mutation carriers have an absolute risk of developing a breast cancer at age 70 years of 60%, which varies from 49% to 63% when taking into account the location of the mutation (in LR1 and outside LR1, respectively). The risk of developing breast cancer in 5 years for a 45-year-old woman is, on average, 7.9% (6.40% in LR1 and 8.30% outside LR1). When taking into account menopausal status, this risk varies from 3.5% for an in-LR1-mutation carrier with menopause at age 45 to 6.2% if menopause occurred 5 years later (i.e., at 50 years old), reaching 12.7% if a woman is postmenopausal at age 50 and, now, carries a mutation outside LR1.

## Discussion

Our results confirm that late menarche, a long or a short menstrual cycle, over- or underweight, and being postmenopausal were associated with breast cancer risk in *BRCA1/2* mutation carriers, as observed in the general population. New findings are that the association with the menopause was observed only when the mutation was located in the "high-risk" zones of both genes.

Our study has several limitations. First, the power to detect heterogeneity by mutation location was limited and significant results might occur by chance. Second, our results are based on retrospective information obtained from women who opted for *BRCA1* and *BRCA2* mutation screening and genetic testing. One assumption that underlies the method of weighting used in our analyses is that the absolute disease risks are well estimated, and ascertainment is not dependent on the covariates of interest (26). This assumption would be violated if any of the factors under study changed the likelihood that women might opt to undergo genetic testing. We are unaware of any study that has assessed whether a woman's uptake of genetic testing differs according to these factors and we cannot assess this potential bias. Third, because our data used prevalent cases with some women being interviewed a long time after their breast cancer diagnosis, we cannot exclude that our findings are affected by a potential survival bias. Therefore, we performed extra analyses on subsamples of individuals diagnosed or censured within the 5-year period before their interview, with follow-up being counted only during this 5-year period, and could not detect differences in our results.

Our results on OC use do not clarify the controversial results previously published (7, 29–31). Indeed, we did not find an association between current use and breast cancer risk. We found a significant association only among past OC users when the time since last use was between 2 and 5 years, suggesting a transient

**Table 3.** Risk of breast cancer associated with menopausal status and hormone replacement therapy use

| | One woman per family cohort: 39,666 person-years of follow-up | | | | *BRCA1* mutation carriers: 25,045 person-years of follow-up | | | | *BRCA2* mutation carriers: 14,621 person-years of follow-up | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Person-years[a] | Number of cases[a] | HR[b] (95% CI) | *P* | Person-years[a] | Number of cases[a] | HR[c] (95% CI) | *P* | Person-years[a] | Number of cases[a] | HR[c] (95% CI) | *P* |
| Menopausal status | | | | | | | | | | | | |
| Premenopause | 37,362 | 312 | 1.00 | | 23,773 | 198 | 1.00 | | 13,589 | 114 | 1.00 | |
| Postmenopause | 1,367 | 60 | 1.30 (0.85–2.01) | 0.23 | 747 | 36 | 1.63 (1.00–2.64) | 0.05 | 620 | 24 | 0.54 (0.21–1.40) | 0.20 |
| Type of menopause | | | | | | | | | | | | |
| Premenopause | 37,362 | 312 | 1.00 | | 23,773 | 198 | 1.00 | | 13,589 | 114 | 1.00 | |
| Natural | 1,078 | 51 | 1.58 (0.93–2.69) | 0.09 | 574 | 30 | 2.14 (1.16–3.93) | 0.01 | 504 | 21 | 0.54 (0.19–1.52) | 0.24 |
| Surgical | 289 | 9 | 0.88 (0.42–1.84) | 0.73 | 173 | 6 | 0.99 (0.44–2.26) | 0.99 | 116 | 3 | 0.54 (0.11–2.51) | 0.43 |
| Age at natural menopause (y) | | | | | | | | | | | | |
| Premenopause | 37,362 | 312 | 1.00 | | 23,773 | 198 | 1.00 | | 13,589 | 114 | 1.00 | |
| <50 | 470 | 21 | 1.40 (0.78–2.50) | 0.26 | 230 | 13 | 1.79 (0.91–3.54) | 0.09 | 240 | 8 | 0.49 (0.16–1.50) | 0.21 |
| ≥50 | 608 | 30 | 2.46 (1.03–5.86) | 0.04 | 344 | 17 | 4.84 (1.63–14.4) | $<10^{-3}$ | 264 | 13 | 0.67 (0.16–2.72) | 0.57 |
| Time since natural menopause (y) | | | | | | | | | | | | |
| Premenopause | 37,362 | 312 | 1.00 | | 23,773 | 198 | 1.00 | | 13,589 | 114 | 1.00 | |
| <5 | 433 | 26 | 1.61 (0.90–2.86) | 0.11 | 252 | 18 | 2.13 (1.10–4.13) | 0.03 | 181 | 8 | 0.51 (0.16–1.58) | 0.24 |
| 5–9 | 282 | 9 | 1.30 (0.56–3.05) | 0.54 | 149 | 5 | 1.97 (0.76–5.11) | 0.17 | 133 | 4 | 0.43 (0.10–1.92) | 0.27 |
| >9 | 363 | 16 | 2.06 (0.70–6.09) | 0.19 | 173 | 7 | 2.80 (0.46–17.2) | 0.27 | 190 | 9 | 0.76 (0.20–2.84) | 0.68 |
| HRT use | | | | | | | | | | | | |
| Never | 946 | 40 | 1.00 | | 554 | 25 | 1.00 | | 392 | 15 | 1.00 | |
| Current | 343 | 16 | 1.20 (0.61–2.34) | 0.60 | 162 | 10 | 1.48 (0.65–3.35) | 0.35 | 181 | 6 | 0.70 (0.24–2.02) | 0.51 |
| Past | 59 | 3 | 1.51 (0.34–6.73) | 0.59 | 31 | 1 | 1.02 (0.09–11.2) | 0.99 | 28 | 2 | 1.21 (0.23–6.26) | 0.82 |
| Premenopause | 37,261 | 312 | 0.81 (0.51–1.27) | 0.36 | 23,724 | 198 | 0.65 (0.39–1.08) | 0.10 | 13,537 | 114 | 1.75 (0.65–4.68) | 0.27 |
| Duration of HRT use (y) | | | | | | | | | | | | |
| Never | 946 | 40 | 1.00 | | 554 | 25 | 1.00 | | 392 | 15 | 1.00 | |
| ≤5 | 228 | 9 | 0.67 (0.29–1.57) | 0.36 | 126 | 5 | 0.71 (0.25–2.06) | 0.53 | 102 | 4 | 0.63 (0.17–2.24) | 0.47 |
| >5 | 167 | 10 | 2.29 (1.07–4.89) | 0.03 | 67 | 6 | 3.46 (1.53–7.85) | $<10^{-3}$ | 100 | 4 | 0.89 (0.27–2.91) | 0.85 |
| Time since last HRT (y) | | | | | | | | | | | | |
| <1 | 509 | 21 | 1.00 | | 251 | 12 | 1.00 | | 258 | 9 | 1.00 | |
| 2–5 | 49 | 2 | 1.27 (0.20–7.90) | 0.80 | 25 | 1 | 1.02 (0.08–13.2) | 0.99 | 24 | 1 | 1.23 (0.12–13.0) | 0.87 |
| >5 | 32 | 1 | 0.57 (0.05–6.96) | 0.66 | 26 | 0 | | | 6 | 1 | 10.0 (0.3–334) | 0.20 |

[a]Not including missing data.

[b]For menopausal status, type of menopause, age at natural menopause, and time since natural menopause: adjusted for parity (0, 1, 2, ≥3), BMI [<18.5, (18.5–25), >25], HRT use (yes or no), and mutated gene (*BRCA1* or *BRCA2*). For factors related to HRT use: adjusted for parity (0, 1, 2, ≥3), BMI [<18.5, (18.5–25), >25], and mutated gene (*BRCA1* or *BRCA2*).

[c]For menopausal status, type of menopause, age at natural menopause, and time since natural menopause: adjusted for parity (0, 1, 2, ≥3), BMI [<18.5, (18.5–25), >25], and HRT use (yes or no). For factors related to HRT use: adjusted for parity (0, 1, 2, ≥3) and BMI [<18.5, (18.5–25), >25].

augmentation of breast cancer risk especially among *BRCA1* mutation carriers. This transient effect might be an artifact due to presymptomatic events, which might prompt some patients to stop OC use. However, this might also be partially explained by the level of circulating insulin-like growth factor I (IGFI), which has been shown to be associated with breast cancer when elevated (32, 33) and higher among past OC users compared with never or current users (34). However, the relation between IGFI level and OC use seems to be complex and is influenced by different factors, like age at first or last use or the generation of OC. Therefore, further analyses on more detailed and larger datasets are needed before firm conclusions can be drawn.

Even though there is some evidence of a positive association between HRT use and breast cancer risk in the general population (35), this association remains less clear among *BRCA1/2* mutation carriers. So far, the effects of HRT for women carrying a *BRCA1/2* mutation have been investigated in few studies, with inconsistent results (12, 13). Like the findings of Sade and colleagues (13), our results suggest an increased breast cancer risk for a cumulative use of HRT greater than 5 years, but only among *BRCA1* mutation carriers. However, the effect of HRT is difficult to assess because of the lack of postmenopausal women also exposed to HRT in the population of carriers.

Like in the general population (1), we found that *BRCA1* mutation carriers who reported having menarche at age 12 or older had a significantly lower risk of breast cancer than those who had menarche before age 12; this effect was not seen in *BRCA2* mutation carriers. In addition, as reported by Whelan and colleagues (36) in the general population, we observed, that short or long menstrual cycles were associated with a higher risk of breast cancer, especially among *BRCA1* mutation carriers. We found that taller women might be at increased risk of breast cancer as shown in the general population (e.g., ref. 37), particularly those carrying a mutation in *BRCA2*. We also studied the impact of BMI, taking into account menopausal status, which is known to modify the association between overweight and breast cancer risk (1). Moreover, BMI, in terms of obesity or underweight, may be a surrogate for measuring potential alteration in patterns of hormones, including sex hormones, which may influence cancer risk (38). Our results are similar to those often found in the general population, that is, overweight associated with a lower risk of breast cancer among premenopausal women. In addition, we found that underweight was associated with a higher breast cancer risk also among premenopausal women. So far, among the five publications that assessed the effect of BMI in *BRCA1/2* mutation carriers (14, 15, 39–41), only two studies adjusted for or stratified by menopausal status (14, 15). The largest study performed by Kotsopoulos and colleagues (15) focused on changes in body weight. The authors observed that a loss of weight between ages 18 and 30 years was associated with a decreased risk of breast cancer

**Table 4.** Risk of breast cancer associated with BMI and height

| | One woman per family cohort: 39,666 person-years of follow-up | | | | *BRCA1* mutation carriers: 25,045 person-years of follow-up | | | | *BRCA2* mutation carriers: 14,621 person-years of follow-up | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Person-years[a] | Number of cases[a] | HR[b] (95% CI) | *P* | Person-years[a] | Number of cases[a] | HR[c] (95% CI) | *P* | Person-years[a] | Number of cases[a] | HR[c] (95% CI) | *P* |
| BMI[c] | | | | | | | | | | | | |
| 18.5–25 | 27,511 | 275 | 1.00 | | 17,054 | 172 | 1.00 | | 10,457 | 103 | 1.00 | |
| ≥25 | 9,806 | 75 | 0.61 (0.45–0.84) | $<10^{-3}$ | 6,541 | 54 | 0.65 (0.45–0.93) | 0.02 | 3,265 | 21 | 0.55 (0.30–1.01) | 0.05 |
| <18.5 | 2,226 | 29 | 2.09 (1.31–3.35) | $<10^{-3}$ | 1,327 | 14 | 1.66 (0.88–3.13) | 0.12 | 899 | 15 | 4.17 (1.85–9.38) | $<10^{-3}$ |
| Among premenopausal women | | | | | | | | | | | | |
| 18.5–25 | 26,130 | 233 | 1.00 | | 16,267 | 146 | 1.00 | | 9,863 | 87 | 1.00 | |
| ≥25 | 9,021 | 52 | 0.55 (0.38–0.79) | $<10^{-3}$ | 6,118 | 39 | 0.56 (0.37–0.85) | 0.01 | 2,903 | 13 | 0.48 (0.24–0.97) | 0.04 |
| <18.5 | 2,099 | 27 | 2.40 (1.47–3.93) | $<10^{-3}$ | 1,276 | 13 | 1.82 (0.96–3.45) | 0.07 | 823 | 14 | 5.46 (2.49–12.0) | $<10^{-3}$ |
| Among postmenopausal women | | | | | | | | | | | | |
| 18.5–25 | 743 | 35 | 1.00 | | 364 | 20 | 1.00 | | 379 | 15 | 1.00 | |
| ≥25 | 577 | 23 | 0.87 (0.46–1.64) | 0.66 | 361 | 15 | 1.05 (0.49–2.24) | 0.91 | 216 | 8 | 0.76 (0.25–2.32) | 0.63 |
| <18.5 | 36 | 2 | 1.60 (0.54–4.75) | 0.40 | 11 | 1 | 1.13 (0.17–7.42) | 0.90 | 25 | 1 | 1.88 (0.53–6.64) | 0.33 |
| Height (m) | | | | | | | | | | | | |
| <1.55 | 3,915 | 41 | 1.00 | | 2,383 | 24 | 1.00 | | 1,532 | 17 | 1.00 | |
| 1.55–1.60 | 9,777 | 103 | 1.11 (0.70–1.76) | 0.65 | 5,995 | 64 | 1.22 (0.70–2.13) | 0.48 | 3,782 | 39 | 0.91 (0.40–2.09) | 0.83 |
| 1.60–1.65 | 12,112 | 104 | 1.04 (0.65–1.65) | 0.87 | 7,915 | 74 | 1.18 (0.68–2.07) | 0.55 | 4,197 | 30 | 0.85 (0.37–1.99) | 0.72 |
| 1.65–1.70 | 9,006 | 92 | 1.33 (0.82–2.16) | 0.25 | 5,618 | 58 | 1.31 (0.72–2.37) | 0.37 | 3,388 | 34 | 1.67 (0.74–3.81) | 0.22 |
| >1.70 | 4,796 | 39 | 1.49 (0.82–2.69) | 0.19 | 3,074 | 20 | 1.30 (0.62–2.69) | 0.49 | 1,722 | 19 | 3.28 (1.05–10.2) | 0.04 |

[a]Not including missing data.
[b]For BMI: adjusted for parity (0, 1, 2, ≥3), menopausal status (premenopause and postmenopause), and mutated gene (*BRCA1* or *BRCA2*). For BMI among premenopausal women or postmenopausal women: adjusted for parity (0, 1, 2, ≥3) and mutated gene (*BRCA1* or *BRCA2*). For height: adjusted for parity (0, 1, 2, ≥3), age at menarche (<12, 12–15, >15), weight (<55, 55–59, 60–64, >64), and mutated gene (*BRCA1* or *BRCA2*).
[c]For BMI: adjusted for parity (0, 1, 2, ≥3) and menopausal status (premenopause and postmenopause). For BMI among premenopausal women or postmenopausal women: adjusted for parity (0, 1, 2, ≥3). For height: adjusted for parity (0, 1, 2, ≥3), age at menarche (<12, 12–15, >15), and weight (<55, 55–59, 60–64, >64).

at ages 30 to 40 years. Manders and colleagues (14) also stratified by menopausal status and reported no clear effect of BMI among premenopausal women, whereas they found that overweight and weight gain increased the risk of postmenopausal breast cancer.

The effect of oophorectomy has been well studied among *BRCA1/2* mutation carriers, and there is almost no controversy about its protective effect on breast cancer risk (e.g., refs. 16, 17), even after a natural menopause (42). The only study that investigated the effect of a natural menopause among *BRCA1/2* mutation carriers did not find an association, but had a lower percentage of women with a natural menopause (9.4% vs. 12.8% in GENEPSO; ref. 2). Our results are nevertheless in line with studies based on the general population (43).

We also found evidence for heterogeneity in the effect of a natural menopause according to the location of mutation. The increased breast cancer risk associated with a natural menopause was confined in the "high-risk" zones of the genes. We observed previously that the protective effect of parity disappeared in such zones in *BRCA1* (44). These observations are consistent with the hypothesis that truncating mutations located in the N-terminal or the C-terminal regions of genes might lead to residual proteins (by reinitialization of transcription when mutations occur in the N-terminal region or by stabilization of the protein when mutations occur in the C-terminal region), whereas mutations located between these regions might lead to unstable proteins by activation of the nonsense-mediated mRNA decay (45). Indeed, HR2 in *BRCA2* is located in the C-terminal region and corresponds to the

**Table 5.** Risk of breast cancer associated with menopausal status according to *BRCA1* mutation location

| | Outside LR1 (16,690 person-years of follow-up) | | | | In LR1 (5,367 person-years of follow-up) | | | |
|---|---|---|---|---|---|---|---|---|
| ***BRCA1*** | Person-years[a] | Number of cases[a] | HR[b] (95% CI) | *P* | Person-years[a] | Number of cases[a] | HR[b] (95% CI) | *P* |
| Menopausal status | | | | | | | | |
| Premenopause | 15,807 | 133 | 1.00 | | 5,114 | 40 | 1.00 | |
| Postmenopause | 437 | 27 | 2.15 (1.30–3.55) | $<10^{-3}$ | 174 | 3 | 0.47 (0.16–1.39) | 0.17 |
| Type of menopause | | | | | | | | |
| Premenopause | 15,807 | 133 | 1.00 | | 5,114 | 40 | 1.00 | |
| Natural | 312 | 22 | 2.97 (1.67–5.29) | $<10^{-3}$ | 145 | 2 | 0.37 (0.11–1.27) | 0.11 |
| Surgical | 125 | 5 | 1.11 (0.42–2.89) | 0.84 | 29 | 1 | 1.18 (0.21–6.74) | 0.85 |
| Age at natural menopause (y) | | | | | | | | |
| Premenopause | 15,807 | 133 | 1.00 | | 5,114 | 40 | 1.00 | |
| <50 | 121 | 8 | 2.07 (0.96–4.49) | 0.07 | 72 | 0 | | |
| ≥50 | 191 | 14 | 5.31 (2.08–13.6) | $<10^{-3}$ | 73 | 2 | 0.77 (0.24–2.52) | 0.67 |
| Time since natural menopause (y) | | | | | | | | |
| Premenopause | 15,807 | 133 | 1.00 | | 5,114 | 40 | 1.00 | |
| <5 | 150 | 13 | 2.81 (1.39–5.68) | $<10^{-3}$ | 60 | 1 | 0.28 (0.04–2.24) | 0.23 |
| 5–9 | 90 | 4 | 3.20 (1.07–9.62) | 0.04 | 33 | 0 | | |
| ≥9 | 72 | 5 | 3.36 (0.75–15.0) | 0.11 | 52 | 1 | 0.67 (0.14–3.27) | 0.62 |

[a]Not including missing data.
[b]Adjusted for parity (0, 1, 2, ≥3), BMI [<18.5, (18.5–25), >25], and HRT use (yes or no).

**Table 6.** Risk of breast cancer associated with menopausal status according to *BRCA2* mutation location

| | Outside LR2 and HR2 (10,075 person-years of follow-up) | | | | In LR2 (2,281 person-years of follow-up) | | | | | In HR2 (1,137 person-years of follow-up) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***BRCA2*** | **Person-years[a]** | **Number of cases[a]** | **HR[b] (95% CI)** | ***P*** | **Person-years[a]** | **Number of cases[a]** | **HR[b]** | **(95% CI)** | ***P*** | **Person-years[a]** | **Number of cases[a]** | **HR[b] (95% CI)** | ***P*** |
| Menopausal status | | | | | | | | | | | | | |
| Premenopause | 9,391 | 81 | 1.00 | | | | | | | 1,041 | 15 | 1.00 | |
| Postmenopause | 437 | 20 | 0.81 (0.36–1.81) | 0.61 | | | | | | 24 | 3 | 4.01 (1.21–13.2) | 0.02 |
| Type of menopause | | | | | | | | | | | | | |
| Premenopause | 9,391 | 81 | 1.00 | | | | | | | 1,041 | 15 | 1.00 | |
| Natural | 336 | 17 | 0.87 (0.35–2.11) | 0.75 | No case in postmenopause | | | | | 21 | 3 | 4.01 (1.21–13.2) | 0.02 |
| Surgical | 101 | 3 | 0.64 (0.14–2.95) | 0.56 | | | | | | 3 | 0 | | |
| Age at natural | | | | | | | | | | | | | |
| Premenopause | 9,391 | 81 | 1.00 | | | | | | | 1,041 | 15 | 1.00 | |
| <50 | 139 | 5 | 0.61 (0.20–1.92) | 0.40 | | | | | | 19 | 3 | 5.12 (1.60–16.3) | 0.01 |
| ≥50 | 197 | 12 | 1.45 (0.43–4.86) | 0.55 | | | | | | 2 | 0 | | |
| Time since natural menopause (y) | | | | | | | | | | | | | |
| Premenopause | 9,391 | 81 | 1.00 | | | | | | | 1,041 | 15 | 1.00 | |
| <5 | 118 | 6 | 0.66 (0.22–1.97) | 0.45 | | | | | | 11 | 1 | 2.52 (0.17–36.7) | 0.50 |
| 5–9 | 95 | 3 | 0.63 (0.14–2.91) | 0.56 | | | | | | 6 | 1 | 5.57 (1.12–27.7) | 0.04 |
| ≥9 | 123 | 8 | 1.49 (0.49–4.50) | 0.48 | | | | | | 4 | 1 | 5.23 (1.63–16.8) | 0.01 |

[a]Not including missing data.
[b]Adjusted for parity (0, 1, 2, ≥3), BMI [<18.5, (18.5–25), >25], and HRT use (yes or no).

longest conserved region of the *BRCA2* protein (46), which is known to be of major importance for *BRCA2* protein stability thanks to the DSS1 (for "deleted in split hand/split foot protein1") protein-binding region (47). Therefore, mutations in these "high-risk" regions with even partial conservation may lead to truncated but potentially stable proteins. These stable truncated proteins might have either a deleterious gain-of-function or dominant-negative activity and affect gene activities. For example, a perturbation of the cellular antiproliferative function of *BRCA1* (22–24) may have dramatic consequences for mutation carrier women, and even more for those long and highly exposed to estrogens (e.g., late age at natural menopause), with a potential increase in breast cell division and DNA replication, and therefore in cellular transformation and cancer cell proliferation. The 5 years predicted breast cancer risk for a 45-year-old *BRCA1* mutation carrier woman could, therefore, vary according to mutation location and age at menopause: from 3- to 10-fold the baseline breast cancer risk of the general population (48). If confirmed, this might impact on the recommendations.

An interaction between "being exposed" and the location of the mutation was not found for all factors related to estrogen exposure, and this may be due to a lack of power. Women with an elevated estrogen exposure profile could be dramatically affected by an antiproliferative activity lowered because of the location of the mutation. Such a profile, if well defined, may be important for the

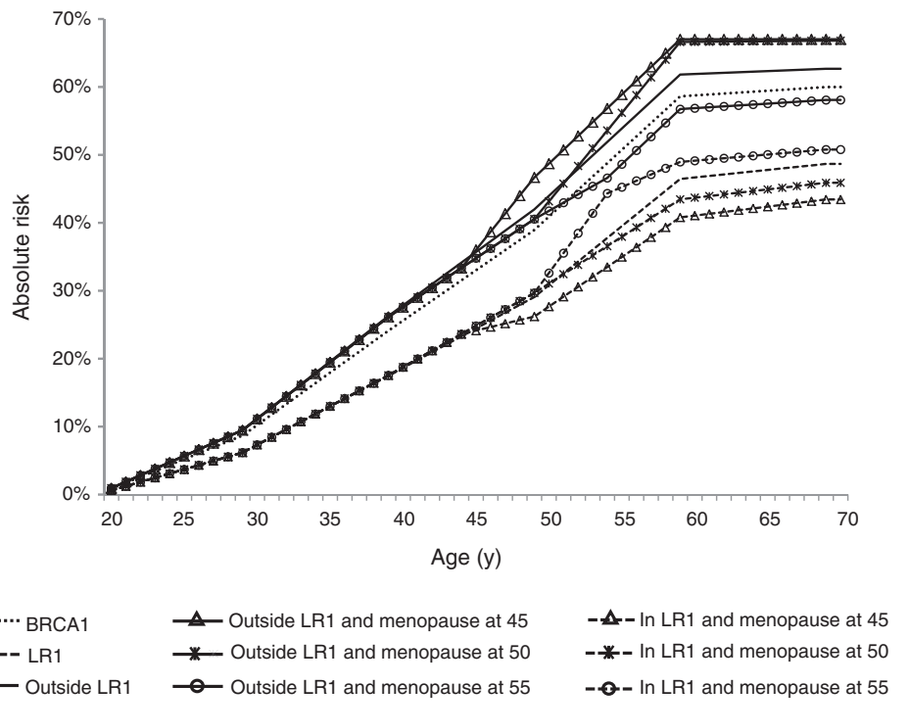


**Figure 1.**
Absolute risk of developing breast cancer for *BRCA1* carrier women according to mutation location and age at menopause (from breast cancer incidence rates estimated by Mavaddat et al.; ref. 28).

clinical management of *BRCA1/2* mutation carriers. Phenotype–genotype correlation in *BRCA1/2* genes may be explained by differential gene activities according to mutation location in interaction with environmental factors. The power to detect such differential effects was limited in our study, but nonetheless allowed us to generate hypotheses to be tested on other, larger datasets.

## Disclosure of Potential Conflicts of Interest

D. Stoppa-Lyonnet is a consultant/advisory board member for Astra-Zeneca. No potential conflicts of interest were disclosed by the other authors.

## Authors' Contributions

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