Breast Cancer Research and Treatment (2019) 174:741–748
https://doi.org/10.1007/s10549-018-05118-3

EPIDEMIOLOGY

Folic acid supplement use and breast cancer risk in *BRCA1* and *BRCA2* mutation carriers: a case–control study

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Received: 5 October 2018 / Accepted: 21 December 2018 / Published online: 2 January 2019

Abstract

Purpose Supplemental folic acid (the more bioavailable and synthetic form of folate) and breast cancer risk in *BRCA* mutation carriers have not been studied. We evaluated folic acid, vitamin B6 and vitamin B12 supplement use, and breast cancer risk among *BRCA* mutation carriers.

Methods In this case–control study, dietary supplement use was collected from *BRCA* mutation carriers living in Canada. Supplement use was categorized as never or ever use. Total average daily supplement use was categorized as never, moderate, and high use based on tertiles. Unconditional logistic regression was used to estimate the odds ratio (OR) and 95% confidence intervals (CI) for supplement use and breast cancer risk.

Results We included 129 breast cancer cases and 271 controls. Women who used any folic acid-containing supplement had a significantly decreased risk of breast cancer compared to women who never used a folic acid-containing supplement (OR 0.45; 95%CI 0.25, 0.79; $P=0.006$). This was significant for *BRCA1* mutation carriers only. The OR for moderate folic acid supplement intake was 0.39; $P=0.01$, and high intake was 0.54; $P=0.09$, compared to never users. Moderate vitamin B12 supplement intake was associated with decreased risk of breast cancer compared to never use (OR 0.48; 95%CI 0.24, 0.96; $P=0.04$).

Conclusions In this first investigation of folic acid supplement use and breast cancer risk in *BRCA* mutation carriers, these findings suggest that moderate folic acid- and vitamin B12-containing supplement use may be protective for *BRCA*-associated breast cancer, particularly among *BRCA1* mutation carriers. Future studies with larger samples and prospective follow-up are needed.

Keywords Folic acid · Multivitamin · Supplements · *BRCA* · Breast cancer

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10549-018-05118-3) contains supplementary material, which is available to authorized users.

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Introduction

In North America, fortification of the food supply with folic acid was mandated in 1998 as a public health initiative to prevent neural tube defects [1]. In addition to fortification, the Centers for Disease Control and Prevention (CDC), the World Health Organization (WHO) and Health Canada strongly recommend that women supplement with at least 400 mcg of folic acid per day, 2 or 3 months prior to pregnancy and into the postpartum period [2–4]. These practices, along with the increased popularity of supplement use in North America, have led to a significant decline in neural tube defects and a rise in circulating folate concentrations across the population [5, 6].

Studies investigating the role of folate in breast cancer development have overall been inconsistent [7, 8]. Folic acid supplementation has been demonstrated to protect or promote the development of cancer, depending on the stage of cell transformation at the time of intervention [9]. Given the role of these proteins in DNA damage repair, adequate folate intake may be necessary for the maintenance of genomic stability [9, 10]. Alternatively, high levels of folic acid supplement use may promote the progression of any possible undiagnosed (pre)neoplastic lesions [9, 10]. An important population of interest are women at high-risk of developing breast cancer due to an inherited *BRCA1* or *BRCA2* mutation [11, 12]. In a small prospective study, we reported that *BRCA* mutation carriers with high circulating plasma folate concentrations had a significant 3.2-fold increased risk of developing breast cancer compared to those with low plasma folate concentrations (95% CI 1.03, 9.92; $P = 0.04$) [13]. Whether or not folic acid supplement use influences breast cancer risk in this high–risk population is not known.

In a case–control study, we evaluated the association between folic acid-containing supplement use and risk of breast cancer among 400 women with a *BRCA1* or *BRCA2* mutation living in Canada. We also investigated the association of vitamin B6 and vitamin B12 supplement use and risk of breast cancer as these vitamins play an important role in DNA synthesis and methylation reactions, similar to folate [9, 14, 15].

Methods

Study population

Eligible study subjects in this case–control study included women with a *BRCA1* or *BRCA2* mutation, as previously described [16]. We identified women aged 18–70 with a confirmed *BRCA1* or *BRCA2* mutation from 10 participating centers across Canada (where mandatory fortification practices exist) who initially sought genetic testing because of a personal or family history of breast and/or ovarian cancer. All study subjects received genetic counseling and provided written informed consent for study participation. Mutation detection was conducted using a range of techniques, but all nucleotide sequences were confirmed by direct sequencing of DNA. The study was approved by the institutional ethics review boards of the host institutions.

Data collection

Each study subject completed a standardized questionnaire at the time of enrolment from 1994 to 2016. Questionnaires collected information on family, medical and reproductive histories including specific questions on preventative mastectomy, history of cancer, parity, height (cm), weight (kg), average number of alcoholic drinks consumed per week and regular smoking habits.

Ascertainment of breast cancer cases was self-reported by participants in the study questionnaire. Cases consisted of first primary invasive breast cancers. Pathology was confirmed for 50% of the reported breast cancers by review of pathology reports. Although several pathology reports were missing, prior studies have demonstrated high sensitivity of self-reported invasive breast cancer diagnosis [17].

Dietary supplement use was collected by a separate, open-ended questionnaire that was distributed to Canadian participants across seven provinces from September 2014 to September 2016. The dietary supplement use questionnaire collected detailed information on supplement use from the age of 18, including any supplements taken during pregnancy. The questionnaire asked about the type of supplement used (i.e., multivitamins or single nutrient supplements), brand name of supplement used, frequency of use per week, dose of supplement and the duration of use.

Total average daily folic acid, vitamin B6 and vitamin B12 supplement use was calculated from any supplements which contained the respective vitamin, either in multivitamins or single nutrient supplements. For example, total folic acid supplement use was derived from folic acid-specific supplements, vitamin B-complexes, multivitamins, and prenatal supplements. The total average daily B vitamin use for the duration of the study was calculated as:

$$\sum \text{dose of B vitamin per pill} \times (\text{frequency of use per week}/7 \text{ days a week}) \times (\text{years of supplement used}/\text{total study years})$$

If information on specific doses of folic acid, vitamin B6 or vitamin B12 from either multivitamins or single nutrient supplements were missing, vitamin doses were derived from the manufacturer's label. If neither the dose nor brand name were indicated, default doses were assigned for each supplement on the basis of the most common doses found in our study (i.e., 400 mcg for folic acid, 100 mg for vitamin B6, and 1000 mcg for vitamin B12 supplements). For multivitamins, we used the formulation from the most commonly used multivitamin brand in Canada, Jamieson (400 mcg of folic acid, 3 mg of vitamin B6, and 12 mcg of vitamin B12). Similarly, we used the Jamieson brand formulation for vitamin B-complex supplements (400 mcg of folic acid, 50 mg of vitamin B6, and 50 mcg of vitamin B12). Prenatal supplements without indicated doses or brand names were derived from the most popular prenatal supplement brand in Canada, Materna (1000 mcg of folic acid, 1.9 mg of vitamin B6, and 2.6 mcg of vitamin B12). If any information regarding frequency or years of supplement used was missing, the observation was removed from the analysis. The validity of self-reported dietary supplement use was previously shown to be good for multivitamins, B-complexes, and folic acid when compared with manufacturers labels (kappa 0.66–0.92) [18]. No dietary information was collected in this longitudinal study; thus, folic acid, vitamin B6 and vitamin B12 intake from only supplements was evaluated.

Subject selection

A total of 512 out of 910 (56%) eligible participants completed and returned the supplemental questionnaire. For the current analysis, subjects were excluded if they had a history of cancer prior to the diagnosis of breast cancer ($n=75$), had a previous bilateral mastectomy ($n=10$), were missing information on *BRCA* mutation type ($n=8$), or were missing information on supplement use ($n=19$). After exclusions, 400 women were included in the current study. Case subjects were women with invasive breast cancer ($n=129$), and controls were women who were not diagnosed with breast cancer ($n=271$). A flowchart of the subject selection is outlined in Fig. 1.

Statistical analyses

A case–control analysis was performed to evaluate the association between vitamin B-containing supplement use and risk of breast cancer. Dietary supplement use and relevant covariates were censored with a lag period of 1 year prior to the diagnosis of breast cancer in the cases. Prenatal, multivitamin, and folic acid supplement use was categorized as never or ever use. Total average daily folic acid, vitamin B6 and vitamin B12 supplement use was categorized as never use, moderate use, and high use based on the distribution of B vitamin supplement intake of the entire cohort using tertiles.

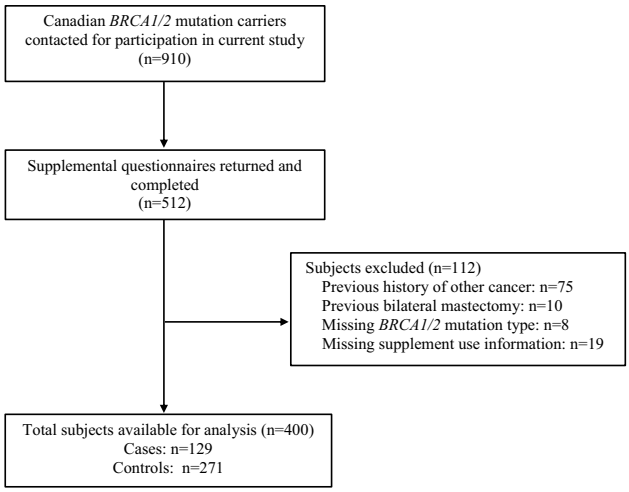

Fig. 1 Flow diagram of the subject selection for the evaluation of dietary supplement use and risk of breast cancer

The student's *t* test and Chi-square test were used to evaluate differences between the baseline continuous and categorical variables, respectively. Unconditional logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) of breast cancer associated with supplement use (never/ever and total average daily use). The basic model was adjusted for age (continuous) and *BRCA* mutation type (*BRCA1* or *BRCA2*). The multivariable model was additionally adjusted for BMI (continuous), parity (0, 1–2, ≥ 3), alcohol consumption (none, 0–3, 4–9, or 10–20 drinks/week), and regular smoking history (never or ever). Missing BMI values were imputed as the median BMI of the study population ($n=8$). Smoking ($n=3$) and alcohol consumption ($n=4$) was imputed as the mode [19]. One study subject carried both a *BRCA1* and *BRCA2* mutation and was categorized as a *BRCA1* mutation carrier.

Effect modification of dietary supplement use and breast cancer risk was investigated by stratifying the results by alcohol intake (never or ever), menopausal status at diagnosis (yes or no), *BRCA* mutation type (*BRCA1* or *BRCA2*), and mandatory folic acid fortification year (< 1998 or ≥ 1998). The statistical significance of the interaction terms was determined using the likelihood ratio test.

All analyses were conducted using SAS version 9.4 software (SAS Institute, Cary, NC). All *P* values were 2-sided and were considered statistically significant if $P \leq 0.05$.

Results

Subject characteristics

Characteristics of the 400 study subjects are presented in Table 1 by case–control status. On average, cases had a

Table 1 Characteristics of *BRCA* mutation carriers included in the study by breast cancer cases and controls

Characteristic	Cases ($n=129$)	Controls ($n=271$)	P value[a]
Age at breast cancer diagnosis (year)	41.3 ± 8.2[b]	–	–
Age (year)[c]	41.3 ± 8.3	42.6 ± 12.3	0.21
Mutation (%)			0.09
BRCA1	62.8	53.9	
BRCA2	37.2	46.1	
BMI (kg/m^2)	26.4 ± 5.7	24.9 ± 4.7	0.007
Age at menarche (year)	12.4 ± 1.4	12.6 ± 1.4	0.13
Parity (%)			0.0004
0	18.6	32.8	
1–2	62.0	41.3	
>3	19.4	25.8	
Prophylactic bilateral oophorectomy (%)	7.0	11.5	0.17
Oral contraceptive use (%)	84.6	81.1	0.40
Alcohol intake (%)			0.53
None	24.0	19.2	
0–3 Drinks/week	58.1	57.9	
4–9 Drinks/week	14.0	18.8	
10–20 Drinks/week	3.9	4.1	
Regular smoker (%)			0.06
Ever	45.7	35.8	
Never	54.3	64.2	
Multivitamin supplement use (%)	24.0	32.8	0.08
Prenatal supplement use (%)[d]	46.3	54.3	0.19
B-complex supplement use (%)	1.6	5.1	0.10
Folate			
Folic acid-specific supplement use (%)	16.7	16.0	0.88
Total average daily folic acid supplement use (mcg/day)	92.5 ± 147.9	115.7 ± 180.6	0.24
Vitamin B6			
B6-specific supplement use (%)	0.8	0.0	0.15
Total average daily B6 supplement use (mg/day)	0.6 ± 1.9	1.4 ± 6.4	0.08
Vitamin B12			
B12-specific supplement use (%)	0.8	2.7	0.22
Total average daily B12 supplement use (mcg/day)	1.7 ± 4.6	15.5 ± 113.1	0.08

[a]P values were calculated using the Student's t test for continuous variables and the Chi-square test for categorical variables

[b]All such values are mean ± SD

[c]Age at breast cancer diagnosis for cases or year of baseline questionnaire for controls

[d]Only among women with a history of pregnancy (except if ending in abortion), $n=296$

higher BMI compared to controls (26.4 kg/m^2 vs. 24.9 kg/m^2; $P=0.007$), and a smaller proportion of cases were nulliparous (18.6% vs. 32.8%, $P=0.0004$). Although not significant, cases were more likely to have a history of smoking (45.7% vs. 35.8%; $P=0.06$).

Case subjects were less likely to have used multivitamins compared to controls (24.0% vs. 35.8%; $P=0.08$). Cases had a lower total average daily intake of vitamin B6 and vitamin B12 supplements compared to controls (0.6 mg/day vs. 1.4 mg/day for vitamin B6; $P=0.08$ and 1.7 mcg/d vs. 15.5 mcg/day for vitamin B12; $P=0.08$). A very small percentage of women took vitamin B-complex (3.8%), vitamin B6-specific (0.25%), or vitamin B12-specific (2.0%) supplements, limiting the analyses of these specific vitamin supplements in the study. The total average folic acid supplement use from multivitamins and other supplements ranged from 0 to 1239.17 mcg/day over all study subjects. The total average daily vitamin B6 supplement use ranged from 0 to 77.68 mg/day, and the total average daily vitamin B12 supplement use ranged from 0 to 1566.56 mcg/day (data not shown in table).

Supplement use and risk of breast cancer

The association between supplement use and breast cancer risk in *BRCA* mutation carriers is presented in Table 2. There was no association between multivitamin use or folic acid-specific supplement use and breast cancer risk.

Among women with a history of pregnancy, past use of prenatal supplements taken for the purposes of pregnancy was associated with a 43% decreased risk of breast cancer compared to never use of prenatal supplements in the basic model adjusted for age and *BRCA* mutation type (OR 0.57; 95% CI 0.34, 0.95; $P=0.03$). This association was attenuated after further adjusting for BMI, parity, alcohol, and smoking (OR 0.60; 95% CI 0.35, 1.02; $P=0.06$).

Women who have used any folic acid-containing supplement (such as folic acid, vitamin B complex, multivitamin, or prenatal supplement) had a nonsignificant decreased risk of breast cancer compared to women who never used supplements in the basic model (OR 0.81; 95% CI 0.50, 1.29; $P=0.37$). After adjusting for parity and other potential confounders, the multivariable model showed a significantly decreased risk of breast cancer among women who used folic acid-containing supplements compared to women who never used supplements (OR 0.45; 95% CI 0.25, 0.79; $P=0.006$). Given the magnitude of the change in the odds ratio estimate after multivariable adjustment, the effects of the confounding variables were estimated individually. Parity was found to be the only significantly confounding risk factor in the analysis (Supplementary Table S1). As expected, the prior use of folic acid supplements increased with increasing parity. After stratification of the analysis by parity, we observed consistent differences in the frequency of folic acid supplement use between cases and controls in all strata (Table 3). Combining the three odds ratios using

Table 2 OR and 95% CI of breast cancer risk by supplement use in *BRCA* mutation carriers

Supplement use	Cases/controls	OR (95% CI) basic model[a]	*P* value	OR (95% CI) multivariable model[b]	*P* value
Multivitamin use					
Never	92/168	Ref.	Ref.	Ref.	Ref.
Ever	29/82	0.66 (0.40, 1.08)	0.10	0.79 (0.46, 1.33)	0.37
Prenatal supplement use[c]					
Never	58/86	Ref.	Ref.	Ref.	Ref.
Ever	50/102	0.57 (0.34, 0.95)	0.03	0.60 (0.35, 1.02)	0.06
Folic acid-specific supplement use[d]					
Never	105/225	Ref.	Ref.	Ref.	Ref.
Ever	21/43	1.06 (0.60, 1.89)	0.83	0.78 (0.42, 1.43)	0.42
Any folic acid-containing supplement use[e]					
Never	39/70	Ref.	Ref.	Ref.	Ref.
Ever	84/188	0.81 (0.50, 1.29)	0.37	0.45 (0.25, 0.79)	0.006
Total average daily folic acid supplement use (mcg/day)					
Never	39/70	Ref.	Ref.	Ref.	Ref.
8.56 –≤ 89.29	26/62	0.73 (0.40, 1.34)	0.31	0.39 (0.19, 0.81)	0.01
> 89.29	30/68	0.81 (0.45, 1.45)	0.48	0.54 (0.27, 1.10)	0.09
Total average daily vitamin B6 supplement use (mg/day)					
Never	53/94	Ref.	Ref.	Ref.	Ref.
0.02–≤ 0.20	21/41	0.92 (0.49, 1.72)	0.79	0.59 (0.29, 1.18)	0.13
> 0.20	30/75	0.72 (0.42, 1.24)	0.24	0.62 (0.34, 1.14)	0.12
Total average daily vitamin B12 supplement use (mcg/day)					
Never	52/88	Ref.	Ref.	Ref.	Ref.
0.02–≤ 0.34	22/43	0.88 (0.47, 1.63)	0.68	0.48 (0.24, 0.96)	0.04
> 0.34	29/74	0.67 (0.39, 1.17)	0.16	0.61 (0.33, 1.12)	0.11

Unconditional logistic regression was used to estimate the OR and 95% CI

[a]Adjusted for age (continuous) and *BRCA* mutation type (*BRCA1* or *BRCA2*)

[b]Adjusted for age (continuous), *BRCA* mutation type (*BRCA1* or *BRCA2*), BMI (continuous), parity (0, 1–2, ≥ 3), alcohol consumption (none, 0–3, 4–9, or 10–20 drinks/week), regular smoker (ever or never)

[c]Only among women with a history of pregnancy (except if ending in abortion), $n=255$

[d]Supplements only include folic acid supplements

[e]Supplements include anything containing folic acid such as folic acid, vitamin B complex, multivitamins, and prenatal supplements

Table 3 Breast cancer risk by folic acid-containing supplement use stratified by parity

Supplement use	Cases/controls	OR (95% CI)	*P* value
Any folic acid-containing supplement use[a]			
Nulliparous			
Never	15/45	Ref.	Ref.
Ever	6/37	0.49 (0.17, 1.38)	0.18
1–2 Live births			
Never	15/13	Ref.	Ref.
Ever	63/98	0.56 (0.25, 1.25)	0.16
> 3 Live births			
Never	9/12	Ref.	Ref.
Ever	15/53	0.38 (0.13, 1.07)	0.07

Unconditional logistic regression was used to estimate the OR and 95% CI

[a]Mantel–Haenszel summary odds ratio = 0.48 (95% CI 0.28, 0.84; $P = 0.008$)

the Mantel–Haenszel test for trend, the summary odds ratio was 0.48 (95% CI 0.28, 0.84; $P = 0.008$).

Women with moderate total average folic acid supplement intake (8.56–≤ 89.29 mcg/day) had a significantly decreased risk of breast cancer compared to never users of folic acid-containing supplements (OR 0.39; 95% CI 0.19, 0.81; $P = 0.01$). Women with the highest intake of total folic acid supplement use (> 89.29 mcg/day) had a nonsignificant decreased risk of breast cancer compared to never users (OR 0.54; 95% CI 0.27, 1.10; $P = 0.09$). *BRCA* mutation carriers with moderate total average vitamin B12 supplement intake (0.02–≤ 0.34 mcg/day) had a decreased risk of breast cancer compared to women who never used a vitamin B12-containing supplement (OR 0.48; 95% CI 0.24, 0.96; $P = 0.04$); however, there was no significant association between the highest intake of total vitamin B12 supplement use (> 0.34 mcg/day) and breast cancer risk compared to never users (OR 0.61; 95% CI 0.33, 1.12; $P = 0.11$). There was no association between total average daily vitamin B6-containing supplement use and breast cancer risk.

Effect modification

BRCA mutation type (i.e., *BRCA1* vs. *BRCA2*) significantly modified the relationship between any folic acid-containing supplement use and risk of breast cancer (*P*-interaction = 0.04) (Table 4). Ever use of any folic acid-containing supplement was associated with decreased breast cancer risk among *BRCA1* mutation carriers compared to never users (OR 0.30; 95% CI 0.14, 0.65 $P = 0.003$), but not among women with a *BRCA2* mutation (OR 0.79; 95% CI 0.32, 1.95; $P = 0.60$). Menopausal status, alcohol intake, and year of mandatory fortification did not significantly modify the association between folic acid-containing supplement use and breast cancer risk (data not shown). Given the small sample size of our stratified analyses, the findings should be interpreted with caution.

Discussion

In this detailed analysis of vitamin B supplement use and breast cancer risk, we observed a decreased risk of breast cancer among women with a *BRCA1* mutation who used folic acid-containing supplements compared to women who never used supplements containing folic acid. No effect was observed for women with a *BRCA2* mutation. The protective association was similar for moderate total average folic acid and vitamin B12 intake. Although limited by a small sample size and retrospective data collection, these findings suggest a potential beneficial role of moderate supplement use containing folic acid or vitamin B12. To our knowledge, this study represents the first such report.

We previously reported in a prospective study, which included 163 Canadian *BRCA* mutation carriers and

Table 4 OR and 95% CI of breast cancer risk by any folic acid-containing supplement use stratified by *BRCA* mutation type

Supplement use	Cases/controls	OR (95% CI) basic model[a]	*P* value	OR (95% CI) multivariable model[b]	*P* value
Any folic acid-containing supplement use					
BRCA1					
Never	27/34	Ref.	Ref.	Ref.	Ref.
Ever	15/36	0.59 (0.32, 1.08)	0.09	0.30 (0.14, 0.65)	0.003
BRCA2					
Never	12/36	Ref.	Ref.	Ref.	Ref.
Ever	11/26	1.30 (0.61, 2.79)	0.50	0.79 (0.32, 1.95)	0.60

Unconditional logistic regression was used to estimate the OR and 95% CI

[a]Adjusted for age (continuous)

[b]Adjusted for age (continuous), BMI (continuous), parity (0, 1 – 2, ≥ 3), alcohol consumption(none, 0 – 3, 4 – 9, or 10 – 20 drinks/week), regular smoker (ever or never)

6.3 years of follow-up, that high circulating plasma folate concentrations were associated with a significantly increased risk of breast cancer (HR 3.20; 95% CI 1.03, 9.92; $P=0.04$) [13]. In contrast, the current study shows a significant inverse association between folic acid supplement intake and breast cancer risk compared to never use. These contrasting findings may be attributed to the relatively small number of women with high levels of folic acid supplement use, limiting our ability to detect a significant effect of high folate intake and breast cancer risk. Furthermore, we only evaluated supplemental folic acid use and not total intake (i.e., dietary and supplement use) which may result in an underestimation of folic acid intake. Despite these contrasting results, the findings from these two studies collectively support prior reports of a nonlinear "U shaped" dose–effect of folate status on cancer risk: moderate folate levels may protect against breast cancer development, while both low and high folate levels may increase risk [20, 21]. In a meta-analysis of prospective studies of folate and breast cancer risk among women in the general population, low (< 153 µg/day) and high (> 400 µg/day) dietary folate intake was associated with an increased breast cancer risk, while mid-range intakes were protective [20, 21]. Although the meta-analysis only included studies of dietary folate intake, our two reports are suggestive of a similar "U-shaped" relationship among women with a *BRCA1* or *BRCA2* mutation.

The dual modulatory role of low and high folate status on carcinogenesis has been proposed to depend on the stage of cell transformation at the time of folic acid supplementation, and dose of supplementation. Folate deficiency in non-transformed cells can result in increased DNA strand breaks, chromosomal instability, and impaired DNA repair [22, 23]. These deficiencies coupled with insufficient *BRCA* protein due to an inherited mutation can propagate and promote neoplastic transformation [9, 23, 24]. Contrastingly, in preneoplastic or neoplastic cells where cells are rapidly replicating, folic acid supplementation fuels the process of replication and proliferation by providing nucleotide precursors, thereby promoting the progression of neoplastic tumor growth and development [9]. Therefore, optimal folate intake may possibly reduce the risk of neoplastic transformation in non-transformed cells and slow the progression of neoplastic cells.

Similar to folate, the relationship between vitamin B12 and vitamin B6 and breast cancer risk has been inconsistent [25]. Overall, evidence in the general population suggests high vitamin B6 and vitamin B12 may be protective against breast cancer; however, the evidence still remains unclear [25]. We found that moderate vitamin B12 supplement use may decrease breast cancer risk compared to women who never used vitamin B12-containing supplements. Since the main source of vitamin B12 supplements among the women in this study was from the multivitamins and not vitamin B12-specific supplements alone, it is difficult to disengage this effect from that of multivitamin or prenatal supplement use. Despite this, we found no significant association between multivitamins, folic acid-specific supplement use or vitamin B6 supplement use and breast cancer risk.

Our study showed that prenatal supplement use during pregnancy was moderately and nonsignificantly associated with decreased breast cancer risk. The role of prenatal or folic acid supplement use during pregnancy and subsequent maternal breast cancer risk has not been well studied; however, one trial reports that pregnant women randomized to 0.2 or 5 mg/day of folic acid supplement use had no increased risk of all-cause or breast cancer-specific mortality compared to placebo [26]. While epidemiologic evidence strongly supports a protective effect of high dietary folate intake among those who consume alcohol, a known folate antagonist [27], we did not observe any significant effect modification by alcohol intake. We found no evidence for effect modification by menopausal status or food fortification year. Given the small sample size of our stratified analyses, these findings should be interpreted with caution.

There are several limitations to the study. We did not collect information on dietary intake, and the analysis was restricted to vitamin B exposure from supplement use only. This importantly underestimates the amount of folic acid intake as it does not account for folic acid intake from fortified foods. The low response rate for the supplemental questionnaires (56%) may also contribute to response bias in the study. This study was also limited by the relatively small sample size, particularly in the stratified analysis, limiting our ability to detect a significant modifiable effect. The retrospective case–control design may subject our data collection to recall bias, as supplement use information was collected from participants after their breast cancer diagnosis. Additionally, our study may be subject to survival bias, as the analysis was restricted to prevalent cases who could be contacted to recall their past supplemental intake. Lastly, the majority of supplements taken were multivitamin mixtures, including prenatal supplements, making it difficult to disentangle the associations of individual B-vitamins alone and breast cancer risk. Despite these limitations, our study had several strengths including detailed collection of dietary supplement use, confounding variables, and a unique study population.

In conclusion, these findings suggest that moderate use of folic acid-containing supplements and vitamin B12-containing supplements may be protective for *BRCA*-associated breast cancer risk, particularly among *BRCA1* mutation carriers. Nevertheless, *BRCA* mutation carriers should stay within the recommended intake levels for folic acid and be cautious of over-supplementation. Future studies investigating total dietary vitamin B intake with a larger sample

size and prospective study design are needed to clarify this relationship.

Funding This study was funded by the Champions of Genetics Grant from the Canadian Gene Cure Foundation in partnership with the CIHR Institute of Genetics and Canadian Cancer Society Research Institute (703058). SJK was supported by a Province of Ontario Graduate Scholarship and the Enid Walker Graduate Student Award in Women's Health Research. JK is the recipient of a Canada Research Chair, tier II, and SAN is the recipient of a Canada Research Chair, tier I.

Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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