Null Results in Brief

No Association between *BRCA2* N372H and Breast Cancer Risk

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Introduction

Truncation mutations in the *BRCA2* gene cause a substantial increase in risk of breast cancer. However, these mutations are rare in the general population and account for little of the overall incidence of sporadic breast cancer. Recently, an intronic single-nucleotide polymorphism (G/A at bp 30,763,310) was also found associated with breast cancer risk (1). One nonsynonymous polymorphism, N372H, has been observed to moderately increase the risk of breast cancer in earlier studies (2, 3). We assessed the risk associated with these polymorphisms in the prospective Nurses' Health Study cohort.

Materials and Methods

Detailed information regarding the design of this nested case-control study (cases, n = 1,313; controls, n = 1,717) has been published previously (4). In brief, 32,826 women were followed for incident disease from time of blood sample collection in 1989 and 1990 up to May 31, 2000. We genotyped a G/A single nucleotide polymorphism at bp 30,763,310 on contig NT_024524.396 (July 2003 assembly, g30763310a, rs206340) recently described by Freedman et al. (1) and the N372H (rs144848) polymorphism in the *BRCA2* gene via TaqMan analysis (Applied Biosystems, Foster City, CA). Taqman primers, probes, and conditions for genotyping assays are available on request. All genotyping was done with laboratory personnel blinded to case-control status of the samples, which included quality control samples for validation. Concordance for quality control samples was 100%. Statistical analysis was carried out in SAS version 8.2 (SAS Institute, Cary, NC). Genotype-specific relative risks were calculated using conditional logistic regression using PROC PHREG, controlling for age of menopause, age at menarche, age at first birth and parity, postmenopausal hormone use, history of benign breast disease, and family history of breast cancer. Tests for deviation from Hardy-Weinberg equilibrium were carried out using PROC ALLELE. Interaction P values were calculated by likelihood ratio testing using a nominal model.

Results

The g30763310a single-nucleotide polymorphism was out of Hardy-Weinberg equilibrium in the controls (P = 0.02), but not in the cases (P = 0.77). The allele frequency in our control group (0.211) is similar to that observed among Whites by Freedman et al. (0.20) and we had 100% concordance among our blinded QC samples, so this departure from Hardy-Weinberg equilibrium is probably due to chance. We observed a nonsignificant increase in risk with the homozygous variant genotype [odds ratio (OR) wt/wt (reference) versus vt/vt, 1.31; 95% confidence intervals (95% CI), 0.89-2.02] with no increase in risk among heterozygotes (Table 1).

We did not observe any deviation from Hardy-Weinberg equilibrium at the N372H polymorphism of the *BRCA2* gene (P = 0.48) among controls. No association was seen between *BRCA2* N372H and breast cancer risk (Table 1). The allele frequency in our control group (0.273) was similar to that seen by Healey et al. (0.221 in Finns to 0.285 in Germans), Spurdle et al. (0.263 in Australians), and Freedman et al. (0.31). To compare with the Spurdle et al. study, which consisted of women under the age of 60, we stratified our analysis based on age of diagnosis <60 (427 cases, 478 controls) or 60+ (858 cases, 1,182 controls). No association was seen in either the codominant [OR NN (reference) versus NH, 1.06; 95% CI, 0.78-1.44; OR NN (reference) versus HH, 0.97; 95% CI, 0.55-1.71] or the recessive model [OR NN and NH (reference) versus HH, 0.94; 95% CI, 0.54-1.63] in women under 60. Similarly, no association was seen upon stratification on menopausal status at diagnosis [premenopausal 109 cases, 107 controls; OR NN (reference) versus NH, 0.85; 95% CI, 0.39-1.89; OR NN (reference) versus HH, 0.49; 95% CI, 0.09-2.45; OR NN and NH (reference) versus HH, 0.53; 95% CI, 0.11-2.57; postmenopausal 1,099 cases, 1,472 controls; OR NN (reference) versus NH, 1.02; 95% CI, 0.85-1.22; OR NN (reference) versus HH, 0.91; 95% CI, 0.66-1.27; OR NN and NH (reference) versus HH, 0.91; 95% CI, 0.66-1.25].

Conclusions

Freedman et al. report an increase in risk of breast cancer associated with the AA genotype of g30763310a [OR GG (reference) versus AA, 1.59; 95% CI, 1.18-2.16]. We had >95% power to detect this association in our data set. However, our confidence intervals include both the point estimate observed by Freedman et al. and 1.00. Therefore, although we were unable to confirm this association, the results of our two studies are not statistically different. We did not see any difference in risk associated with this genotype upon stratification by menopausal status, age at diagnosis, or family history (data not shown).

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Table 1. Association between N372H of *BRCA2* and risk of breast cancer in the Nurses' Health Study Cohort

	Case (%)	Control (%)	RR (95% CI)*
g30763310g	789 (61)	1,029 (61)	1.00 (Reference)
g30763310a	442 (34)	591 (35)	0.98 (0.83-1.15)
a30763310a	59 (4.6)	58 (3.5)	1.31 (0.86-2.01)
N372N	695 (54)	884 (53)	1.00 (Reference)
N372H	501 (39)	647 (39)	0.99 (0.84-1.17)
H372H	89 (6.9)	129 (7.8)	0.88 (0.65-1.19)
N372N/N372H	1,196 (93)	1,532 (92)	1.00 (Reference)
H373H	89 (6.9)	129 (7.8)	0.88 (0.65-1.19)

Abbreviation: RR, relative risk.
*Conditional logistic regression controlled for age of menopause, age at menarche, age at first birth, parity, postmenopausal hormone use, history of benign breast disease, and family history of breast cancer.

We did not observe an association between the N372H polymorphism in *BRCA2* and risk of breast cancer among Caucasian women. The increase in risk observed by Healey et al. is seen comparing HH homozygotes to NN homozygotes [3,459 cases, 2,807 controls in five case-control studies, NN (reference) versus HH OR, 1.31; 95% CI, 1.07-1.61]. It should be noted that only one of five studies reported by Healey et al. showed a statistically significant increase in risk (1,667 cases or 48% or total cases and 1,201 controls or 43% of total controls), although they did not detect any heterogeneity between studies. Spurdle et al. also found significant results following a recessive model, with a 42% increase in risk associated with the NN genotype [1,397 cases, 775 controls, HH and NH (reference) versus NN OR, 1.42; 95% CI, 1.00-2.02]. Whereas our study has only moderate power (power = 0.72 for an OR of 1.40 in the recessive model), the confidence intervals for the main effect in our study comfortably excludes these prior values (OR, 0.88; 95% CI, 0.65-1.19). The main weakness of our study is the sparse numbers of young/premenopausal women, and as such our study is not directly comparable with the previous studies of younger women. However, the prospective nature of the study limits the risk of survivor or selection bias. Whereas we cannot rule out the possibility that *BRCA2* N372H is a risk factor for breast cancer in younger women, our study does not show any association between this polymorphism and breast cancer, particularly for postmenopausal women.

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