Short Communication

The *BRCA2* 372 *HH* Genotype Is Associated with Risk of Breast Cancer in Australian Women Under Age 60 Years[1]

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Abstract

The *BRCA2* N372H nonconservative amino acid substitution polymorphism appears to affect fetal survival in a sex-dependent manner, and the *HH* genotype was found to be associated with a 1.3-fold risk of breast cancer from pooling five case-control studies of Northern European women. We investigated whether the *BRCA2* N372H polymorphism was associated with breast cancer in Australian women using a population-based case-control design. The *BRCA2* 372 genotype was determined in 1397 cases under the age of 60 years at diagnosis of a first primary breast cancer and in 775 population-sampled controls frequency matched for age. Case-control analyses and comparisons of genotype distributions were conducted using logistic regression. All of the statistical tests were two-tailed. The *HH* genotype was independent of age and family history of breast cancer within cases and controls, and was more common in cases (9.2% *versus* 6.5%). It was associated with an increased risk of breast cancer, 1.47-fold unadjusted (95% confidence interval, 1.05–2.07; $P = 0.02$), and 1.42-fold (95% confidence interval, 1.00–2.02; $P = 0.05$) after adjusting for measured risk factors. This effect was still evident after excluding women with any non-Caucasian ancestry or the 33 cases known to have inherited a mutation in *BRCA1* or *BRCA2*, and would explain ~3% of breast cancer. The *BRCA2* N372H polymorphism appears to be associated with a modest recessively inherited risk of breast cancer in Australian women. This result is consistent with the findings for Northern European women.

Introduction

Although there are many hundreds of germ-line mutations in *BRCA1* and *BRCA2* that cause a substantially increased risk of female breast cancer, their rarity means that they explain at most a small percentage of all of the cases. It is possible that one or more of the common polymorphisms in these genes could be associated with a modest risk that would explain, on a population basis, a similar or larger proportion of the disease.

The *BRCA2 N*372*H* nonconservative amino acid substitution polymorphism falls within a region of BRCA2 (residues 290–453) that has been shown to interact with the histone acetyltransferase P/CAF before the transcriptional activation of other genes (1) and appears to affect fetal survival in a sex-dependent manner (2). Newborn females had an excess of heterozygotes and deficit of homozygotes, whereas the opposite was observed in newborn males (2). Pooling of genotype data from five large case-control studies of relatively early onset female breast cancer conducted in the United Kingdom, Germany, and Finland also showed deviation from HWE;[3] controls had a deficit of homozygotes, whereas cases had an excess of homozygotes. Consequently, the *H* allele was associated with a 1.3-fold recessively inherited risk that would explain 2% of breast cancers occurring in that age range in those populations (2). We have undertaken a study to investigate the breast cancer risk associated with the *BRCA2* genotype defined by this polymorphism in Australian women of a similar age.

Materials and Methods

Subjects. A population-based case-control-family study of breast cancer in women under the age of 40 years was carried out in Melbourne and Sydney from 1992 to 1995 (3, 4) and extended from 1996 to 2000 to include women up to age 59 years (5). Age groups were determined by age at diagnosis for cases and by age at invitation to the study for controls. Cases with a diagnosis of first primary breast cancer were identified through the Victorian or New South Wales cancer registries. Controls without breast cancer were selected from the electoral roll (adult registration for voting is compulsory in Australia) using stratified random sampling and frequency

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[3] The abbreviations used are: HWE, Hardy Weinberg equilibrium; CI, confidence interval; OR, odds ratio.

matched for age. Cases, controls, and their relatives (4, 5) were administered a questionnaire to record basic demographics and known or potential risk factors, including family history of breast cancer. Relatives of cases and controls were not included as probands in case-control analyses. Ancestry was assessed by an open-ended question, and country of birth was asked for the respondents, their parents, and grandparents. For the purpose of the subanalyses restricted to Caucasian women, subjects were excluded if any of the ancestry fields mentioned Australian aboriginal, Torres Strait Island, or Maori heritage, or if the country of birth fields mentioned South Pacific, Indian Ocean, Caribbean islands, or Asia.

For each case and control, a detailed family history was systematically recorded for all of the first- and second-degree relatives, checked at interview with their living relatives. Verification based on records was sought (4). Subjects who reported having at least one first- or second-degree female relative with breast cancer were considered to have a "family history."

Interviews were conducted for 1579 of 2304 eligible cases (68.5%) and 1021 of 1531 eligible controls (66.7%). Attrition of cases was attributable to death (1.8%), refusal by surgeon (8.5%), refusal by proband (16.4%), nonresponse by surgeon (1.3%), nonresponse by proband (1.2%), and failure to locate proband (2.3%). Attrition of controls was attributable to refusal (28.2%) and nonresponse (5.1%). Not all of the interviewed cases and controls elected to donate a blood sample for DNA studies. *BRCA2 N*372*H* genotyping was performed on the 1402 cases (88.8% of participating) and 780 controls (76.3% of participating) from whom a DNA sample was available at the time, and genotype results were obtained for 1397 cases and 775 controls (PCR success rate >99.4%). Genotyping was independent of factors shown previously to be associated with breast cancer in this study (4), except genotyped cases were on average 1.7 cm taller than nongenotyped cases ($P = 0.002$), and genotyped individuals were more likely to report a family history than nongenotyped individuals: 32.9% *versus* 22.5% for cases ($P = 0.005$) and 23.9% *versus* 10.6% for controls ($P < 0.001$), but the difference was similar in cases and controls ($P = 0.1$). Genotyped cases ranged in age at diagnosis from 22 to 59 years (mean 41.8; SD 8.7), with 735 (53%) under 40, 328 (24%) 40–49, and 334 (24%) 50–59. Genotyped controls ranged in age at interview from 20 to 60 years (mean 40.9; SD 9.0), with 429 (56%) under 40, 173 (22%) 40–49, and 173 (22%) >50 years.

To date, mutation testing has been carried out on 662 (47.4%) genotyped cases, restricted almost exclusively to those diagnosed before age 40 years, and 33 cases have been found to carry a deleterious mutation in *BRCA1* or *BRCA2*. Methods of detection included: (*a*) manual sequencing of all coding and flanking intronic sequences of *BRCA1* and *BRCA2* in cases reporting a family history of two or more first- or second-degree relatives and in an additional 100 cases without such family history; and (*b*) in the remainder of cases screened, protein-truncation testing covering the large exons (~70% of the coding regions) and specific screening for the three ancestral Ashkenazi mutations and *BRCA1* duplication 13 (6, 7).[4] Given that the proportion of mutation carriers is likely to be lower in cases with later onset and that our detection methods are unlikely to have missed much more than 30% of carriers, we have probably identified more than half of all of the carriers among the cases.

Approval of this study was obtained from the ethics committees of The University of Melbourne, the New South Wales Cancer Council, The Anti-Cancer Council of Victoria, and The Queensland Institute of Medical Research. Written informed consent was obtained from all of the participating subjects.

Molecular Analysis. Collection of peripheral blood and DNA extraction have been described previously (8). The *BRCA2* N372H *T* to *G* polymorphism was detected using the ABI Prism 7700 Sequence Detection System using Taqman primers, probes, and methodology as described by Healey *et al.* (2). Genotype analysis was performed on amplified samples using the ABI PRISM 7700 software, using the standard procedures for automated allelic discrimination described previously (9).

Statistical Methods. The HWE assumption was assessed using standard methods. The association between *BRCA2 N*372*H* genotype and risk of breast cancer was assessed using unconditional multiple logistic regression with and without adjustment for measured covariates (including family history and height) and after dominant, codominant, multiplicative, and recessive models of risk. Logistic regression with genotype as outcome was used to compare genotype distribution within cases and within controls, in regard to age-group (categorized as 20–29, 30–34, 35–39, 40–44, 45–49, 50–54, and 55–59 years), family history of breast cancer, country of birth (Australia *versus* elsewhere), education level, marital status, number of live births, height, weight, age at menarche, and oral contraceptive use (see Ref. 4 for more details on the categorization of these variables). A recessive mode of inheritance was assumed for this analysis given the findings of Healey *et al.* (2) and those described below in the "Results" section for the different models of effects per genotype. All of the statistical tests and *P*s were two-tailed and, after convention, statistical significance was taken as a nominal *P* of <0.05. SPSS (version 9.0), Epistat, STATA, and Ottutil software was used for the statistical analyses.

Results

Table 1 shows the *BRCA2 N*372*H* genotype distributions for cases and controls in total and stratified by family history of breast cancer or age. The *H* allele frequency (95% CI) was 0.288 (0.272–0.305) in all of the cases and 0.287 in cases without a known mutation, similar to 0.263 (0.241–0.285) in controls ($P = 0.08$), and did not differ by age or family history within cases (with or without known mutation carriers) or within controls ($P > 0.1$).

Tests of HWE showed that, although there was a deficit of *HH* genotypes in controls (50 observed *versus* 53.7 expected), as there was in the female control data previously reported (2), this was not incompatible with chance ($P = 0.5$). There was an excess of *HH* genotypes (129 *versus* 116.3) in cases ($P = 0.1$), and this excess was slightly greater after removal of known mutation carriers (125 *versus* 111.9; $P = 0.08$).

Similarly, the proportion with the *HH* genotype did not differ by age or family history, or by any of the measured potential confounders listed under "Materials and Methods" within cases or within controls (all $P > 0.1$). Case-control analyses are shown in Table 2, with results for the different models of effects per genotype. Under codominant inheritance, the *HH* genotype was associated with an increased risk of 1.49-fold ($P = 0.02$) over the *NN* genotype, but there was

[4] M. Southey and J. Hopper, unpublished observations.

Table 1 *BRCA2* N372H genotype distribution in breast cancer cases and controls

	Total						Family history[a]						No family history						<40 years				≥40 years			
	Cases		Cases - no mutations[b]		Controls		Cases		Cases - no mutations[b]		Controls		Cases		Cases - no mutations[b]		Controls		Cases		Controls		Cases		Controls	
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Genotype																										
NN	720	(51.5)	707	(51.9)	417	(53.8)	228	(49.7)	221	(50.0)	92	(49.7)	492	(52.5)	486	(52.7)	325	(55.1)	388	(52.8)	219	(51.0)	332	(50.2)	198	(57.2)
NH	548	(39.2)	532	(39.0)	308	(39.7)	192	(41.8)	184	(41.6)	85	(45.9)	356	(38.0)	348	(37.7)	223	(37.8)	282	(38.4)	181	(42.2)	266	(40.4)	127	(36.7)
HH	129	(9.2)	125	(9.2)	50	(6.5)	39	(8.5)	37	(8.4)	8	(4.3)	90	(9.6)	88	(9.5)	42	(7.1)	65	(8.9)	29	(6.8)	64	(9.7)	21	(6.1)
Total	1397		1364		775		459		442		185		938		922		590		735		429		662		346	
H allele frequency	0.288		0.287		0.263		0.294		0.292		0.273		0.286		0.284		0.260		0.280		0.279		0.298		0.244	
95% CI	0.272–0.305		0.270–0.304		0.241–0.285		0.265–0.324		0.261–0.322		0.228–0.318		0.265–0.306		0.264–0.305		0.235–0.285		0.257–0.303		0.249–0.309		0.273–0.322		0.212–0.276	

[a] Family history defined as any reported first- or second-degree relative with breast cancer.

[b] Excluding carriers of *BRCA1* or *BRCA2* deleterious mutations. There was 1 individual with a *BRCA1* and *BRCA2* mutation, and 19 *BRCA1* and 13 *BRCA2* carriers. There was no difference in genotype distribution between *BRCA1* and *BRCA2* carriers ($P = 0.6$), and the *HH* genotype was found in only 1 *BRCA1* and 2 *BRCA2* carriers. There was also no difference in genotype distribution between all *BRCA1/2* carriers and noncarriers ($P = 0.4$).

no increased risk for the *NH* genotype. Of all of the models, the recessive model gave the most parsimonious fit. Compared with the baseline of pooled *NN* and *NH* genotypes, the *HH* genotype was associated with an increased risk of 1.47-fold when unadjusted ($P = 0.02$) and virtually unchanged at 1.42-fold when adjusted for the listed measured risk factors ($P = 0.05$). Results were marginally different after exclusion of known carriers of deleterious *BRCA2* or *BRCA2* mutations (Table 2). Results were also little different when analyses were restricted to the 1256 cases and 652 controls of Caucasian ancestry, as defined above (see "Subjects"). Under the codominant model, the crude OR (95% CI) was 1.05 (0.86–1.29) for the *NH* genotype ($P = 0.6$) and 1.53 (1.05–2.22) for the *HH* genotype ($P = 0.03$), whereas for the recessive model the OR for the *HH* genotype was 1.50 (1.04–2.15; $P = 0.03$). In addition, the association between the *BRCA2* polymorphism and breast cancer risk was independent of family history ($P = 0.4$): the crude OR (95% CI) for the *HH* genotype under the recessive model was 2.05 (0.94–4.48) for subjects reporting a family history ($P = 0.07$) and 1.38 (0.95–2.03) for those without ($P = 0.09$).

Discussion

The *HH* genotype defined by the *BRCA2 N*372*H* polymorphism appears to be associated with a modest increased risk of breast cancer in Australian women of about 1.4–1.5-fold, similar in magnitude to the 1.3-fold effect found from the combined analysis of British, German, and Finnish women (2). Whether such an effect is because of a direct causative role of this variant, or linkage disequilibrium with a deleterious variant in *BRCA2* or another nearby gene, is as yet unknown.

In our study, as in three of the five studies (2), cases of early onset were predominant. Using all of the controls as a comparison group, the crude OR for the *HH* genotype was 1.41 (0.94–2.11) for women under 40 years, similar to the 1.55 (1.04–2.32) for women ages 40–59 years ($P = 0.3$). That is, there was no evidence that the allele frequency or the magnitude of recessively inherited risk associated with the *H* allele varied across the age range of our sample. Similarly, the allele frequency and the recessively inherited risk did not vary across strata defined by family history, suggesting that differences in uptake of subjects in molecular studies by family history did not confound results.

As in the Northern European control samples and the newborn United Kingdom females, our controls had a deficit of *HH* genotypes, and we also observed an excess of *HH* genotypes in cases, as observed previously in Northern European cases. Furthermore, we found evidence for an increased risk of breast cancer associated with recessive inheritance of the *BRCA2 N*372*H* polymorphism, despite only 25% power to detect an effect of 1.3-fold or more with our study of >2000 subjects. That is, a null finding would not have been strong evidence against the hypothesis. Our results support the findings of the hypothesis-generating study of 3275 cases and 3014 controls. This modest increase in risk would explain about the same percentage of disease because of the rare high-risk mutations in *BRCA1* and *BRCA2*. We now intend to genotype relatives for this *BRCA2* polymorphism to provide an independent test of this apparent disease association, as we have done previously for a common variant in the *CYP17* gene (9).

Table 2 Risk of breast cancer associated with the *BRCA2* N362H genotype according to different models of inheritance[a]

	Including carriers of *BRCA1* or *BRCA2* deleterious mutations				Excluding carriers of *BRCA1* or *BRCA2* deleterious mutations			
	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
Codominant inheritance								
NN	Reference		Reference		Reference		Reference	
NH	1.03(0.86–1.24)	0.7	1.02(0.84–1.24)	0.8	1.01(0.84–1.22)	0.9	1.03(0.85–1.24)	0.8
HH	1.49(1.05–2.11)	0.02	1.43(1.00–2.05)	0.05	1.46(1.03–2.07)	0.04	1.40(0.98–2.01)	0.07
Multiplicative risk per *H* allele	1.13(0.99–1.30)	0.07	1.12(0.97–1.29)	0.1	1.12(0.98–1.29)	0.1	1.11(0.96–1.28)	0.2
Dominant inheritance								
NN	Reference		Reference		Reference		Reference	
NH/HH	1.10(0.92–1.31)	0.3	1.08(0.90–1.30)	0.4	1.08(0.91–1.29)	0.4	1.07(0.89–1.28)	0.5
Recessive inheritance								
NN/NH	Reference		Reference		Reference		Reference	
HH	1.47(1.05–2.07)	0.02	1.42(1.00–2.02)	0.05	1.46(1.04–2.06)	0.03	1.42(1.00–2.02)	0.05

[a] Adjusted ORs were adjusted for age, country of birth, state, education, marital status, number of live births, height, weight, age at menarche, oral contraceptive use, and for reported family history of breast cancer (first- or second-degree).

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