Polymorphisms in BRCA1 and BRCA2 and Risk of Epithelial Ovarian Cancer

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ABSTRACT

Purpose: **Because inherited BRCA1or BRCA2 mutations strikingly increase ovarian cancer risk, polymorphisms in these genes could represent low penetrance susceptibility alleles. Previous studies of the BRCA2 N372H polymorphism suggested that HH homozygotes have a modestly increased risk of both breast and ovarian cancer. We have examined whether BRCA2 N372H or common amino acid-changing polymorphisms in BRCA1 predispose to ovarian cancer.**

Experimental Design: **A population-based, case control study of ovarian cancer was performed in North Carolina. Cases included 312 women with ovarian cancer (76% invasive and 24% borderline) and 401 age- and race-matched controls. Blood DNA from subjects was genotyped for BRCA2 N372H and BRCA1 Q356R and P871L.**

Results: **There was no association between BRCA2 N372H and risk of borderline or invasive epithelial ovarian cancer. The overall odds ratio (OR) for HH homozygotes was 0.8 [95% confidence interval (CI) = 0.4–1.5] and was similar in all subsets, including invasive serous cases. In addition, neither the BRCA1 Q356R (OR = 0.9, 95% CI 0.5–1.4) nor P871L (OR = 0.9, 95% CI 0.6–1.9) polymorphisms were associated with ovarian cancer risk. There was a significant racial difference in allele frequencies of the P871L polymorphism ($P = 0.64$ in Caucasians, L = 0.76 in African-Americans, $P < 0.0001$).**

Conclusions: **In this population-based, case control study, common amino acid changing BRCA1 and 2 polymorphisms were not found to affect the risk of developing ovarian cancer.**

INTRODUCTION

Germ-line mutations in the BRCA1 or BRCA2 genes strikingly increase lifetime risks of ovarian cancer (10–15% in BRCA2 carriers and 15–30% in BRCA1 carriers; Refs. 1–3). Highly penetrant germ-line BRCA mutations are rare, however, and are carried by <0.5% of individuals in most populations, with the notable exception of Ashkenazi Jews (2.5% carrier rate; Ref. 4). The ability to identify BRCA mutation carriers is an important advance, because these women can consider prophylactic oophorectomy and other approaches aimed at decreasing ovarian cancer mortality (5), but because BRCA mutations are rare, the overall impact on mortality inevitably will be small.

BRCA1 and 2 were identified by focusing on families with multiple early onset breast and/or ovarian cancers, and it is estimated that ~10% of ovarian cancers are attributable to high penetrance mutations in these genes (6–8). However, studies that have compared the incidence of ovarian cancer in identical and fraternal twins have estimated that 22% of cases have a heritable component (9). Although other unknown high penetrance genes may exist, there may be weakly penetrant functional genetic polymorphisms that contribute to the burden of ovarian cancers classified as "sporadic" based on the lack of other cases in a pedigree.

Because BRCA1 and BRCA2 mutations strikingly increase ovarian cancer risk, polymorphisms in these genes are logical candidates in seeking to identify low penetrance susceptibility alleles. In the *BRCA2* gene, N372H is the only amino acid-changing polymorphism with a rare allele frequency of >6%, and an increased risk of breast cancer (crude OR[2] = 1.31) has been reported in HH homozygotes in a large case control study (10). Subsequently, an Australian group also reported that homozygosity for the H allele was associated with increased risks of both breast cancer (OR = 1.42; 95% CI 1.00–2.02; Ref. 11) and ovarian cancer (OR = 1.36; 95% CI 1.04–1.77; Ref. 12). There are five amino acid changing polymorphisms in BRCA1 with rare allele frequencies >5% (Q356R, P871L, E1038G, K1183R, and S1613G; Ref. 13). Some, but not all, previous reports have suggested that these polymorphisms might affect ovarian cancer risk (13–16), but none of these data were derived from population-based case control studies of ovarian cancer. These polymorphisms, with the exception of Q356R, are in significant linkage disequilibrium, and the effect of all of these on ovarian cancer risk can be ascertained by considering only Q356R and P871L (13, 14).

In this study, we examine the association of ovarian cancer risk with BRCA2 N372H and BRCA1 Q356R and P871L in a population-based, case control study in North Carolina.

MATERIALS AND METHODS

Subject Identification and Interview. Study subjects are enrolled through the NCOC study, an ongoing population-based, case control study of newly diagnosed epithelial ovarian cancer. Cases are identified through the North Carolina Central Cancer Registry, a statewide population-based tumor registry, using rapid case ascertainment. Eligible cases are women diagnosed with epithelial ovarian cancer since January 1, 1999, aged 20–74 years, who had no previous history of ovarian cancer, and resided in a 48 county area of North Carolina. Physician permission was obtained before any eligible case was contacted by the study staff. All cases are confirmed by standardized pathological review by the study pathologist. Cases diagnosed with primary epithelial ovarian cancer, either invasive, or of low malignant potential are eligible for the study. The response rate among eligible cases was 85%. Reasons why some patients were not interviewed included death (3.3%), debilitating illness (1.7%), patient refusal (5.4%), physician refusal (5.4%), or the inability to locate the woman (4.4%).

Population-based controls were identified from the same 48 county region as the cases and were frequency matched to the ovarian cancer cases on the basis of race (African-American *versus* non-African-American) and age (5-year age categories) using list-assisted random digit dialing. Although HCFA records (women 65–74 only) lists were used early in the data collection period, enrollment of control women using this method was hindered because of the lack of telephone numbers on the HCFA computer tapes, and the use of HCFA lists was suspended. Potential controls were screened for eligibility and required to have at least one intact ovary. Seventy-three percent of controls identified by random digit dialing who passed the eligibility screening agreed to be contacted and sent additional study information. Among those sent additional study information, the response rate was 70%. The response rate was 39% for HCFA controls from the first 76 eligible subjects identified before the use of HCFA tapes was suspended. The response rate among eligible controls was 67%. Reasons for nonparticipation were refusal (26.1%) and the inability to locate the woman (6.7%). For the purposes of this report, we restricted study subjects to those who either were Caucasian or African-American.

In-person Interview. All women gave written informed consent at the time of the interview. The study protocol was approved by the Duke University Medical Center Institutional Review Board and the human subjects committees at each of the hospitals where cases were identified.

Cases and controls were interviewed in person by trained nurse interviewers, usually in the home of the study subject. A 90-min questionnaire was administered to obtain information on known and suspected ovarian cancer risk factors, including family history of cancer in first and second degree relatives, menstrual characteristics, pregnancy and breastfeeding history, hormone use, and lifestyle characteristics, such as smoking history, alcohol consumption, physical activity, and occupational history. Additionally, anthropometric descriptors (height, weight, waist, and hip circumference) are measured, and a blood sample (30 ml) is collected from each participant at the time of the interview.

Blood Processing. Within 48 h, all blood samples are centrifuged, and the buffy coat, RBCs, and plasma are separated. Germ-line DNA was extracted from peripheral blood using PureGene DNA isolation reagents, according to manufacturer's instructions (Gentra Systems, Minneapolis, MN).

BRCA2 N372H Polymorphism. Direct sequencing of the exon 10 region containing the polymorphic (A→ C) base was performed on extracted leukocyte DNA. A 50-μl PCR reaction was performed using forward primer 5′-CTG AAG TGG AAC CAA ATG ATA CTG A-3′ and reverse primer 5′-AGA CGG TAC AAC TTC CTT GGA GAT-3′, 0.5 ng/μl DNA, 0.5 nmol/liter forward primer, 0.5 nmol/liter reverse primer, 0.2 nmol/liter dNTP, 1.5 mmol/liter $MgCl_2$, (Life Technologies, Inc.), $1 \times$ Amplitaq Gold PCR buffer II, and 0.025 units/μl AmpliTaq Gold DNA polymerase (Roche, Branchburg, NJ). PCR conditions consisted of an initial denaturing step at 95°C for 12 min, 32 cycles of 94°C for 60 s, 55°C for 60 s, and 72°C for 1 min, an extension step at 72°C for 10 min, and then held at 4°C until further processing. Samples were purified using QIAquick 96 vacuum filter plates (Qiagen, Germantown, MD) and finally eluted in 150 μl of 10 mM Tris-Cl (pH 8.5). A sequencing reaction was performed using 1 μl of purified product and 4.4 pmoles of unlabeled forward primer in a BigDye Terminator Cycle Sequencing Reaction as described by the supplier (Applied Biosystems, Foster City, CA). Samples were analyzed on the ABI 377 autosequencer, and sequences were analyzed with Genescan software (Perkin-Elmer).

BRCA1 P871L Polymorphism. Allelic discrimination was performed using the MGB primer/probe TaqMan assay on the ABI Prism 7700. Each 20-μl PCR reaction contained 18 pmoles of forward primer 5′-GGT TTC AAA GCG CCA GTC AT-3′, 18 pmoles of reverse primer 5′-CAC ATT CCT CTT CTG CAT TTC CT-3′, 4 pmoles of "proline" probe 5′-VIC-TGC TC*C* GTT TTC AAA-3′, 4 pmoles of the "leucine" probe 5′-6FAM-TTG CTC *T*GT TTT CAA AT-3′, 10 μl of $2 \times$ TaqMan universal master mix without Amp erase UNG (Applied Biosystems), and 25 ng of extracted leukocyte DNA. Cycling conditions were 95°C for 10 min followed by 40 cycles of 92°C $\times$ 15 s and 60°C $\times$ 60 s. Samples were then read in 96-plate format in the ABI Prism 7700 and analyzed using the ABI Prism 7700 allelic discrimination software.

A fraction of samples were subjected to sequencing to confirm results obtained using the TaqMan assay. A 50-μl PCR reaction was performed using forward primer 5′-CCC AAG GGA CTA ATT CAT GG–3′ and reverse primer 5′-TCT GCA TTT CCT GGA TTT GA–3′, 0.5 ng/μl genomic DNA, 0.5 nmol/liter forward primer, 0.5 nmol/liter reverse primer, 0.2 mmol/liter dNTP, 1.5 mmol/liter $MgCl_2$ (Life Technologies, Inc.), $1 \times$ Life Technologies, Inc. PCR buffer (part #Y02028), and 0.025 unit/μl TaqDNA polymerase (Life Technologies, Inc.; catalogue no. 10342-020). PCR conditions consisted of an initial denaturing step at 95°C for 3 min, 30 cycles of 94°C for 45 s, 57°C for 45 s, and 72°C for 1 min, an extension step at 72°C for 10 min. Samples were held at 4°C until purified using QIAquick 96 vacuum filter plates (Qiagen) and finally eluted in 150 μl of 10 mM Tris-Cl (pH 8.5). A sequencing reaction was performed using 1 μl of purified product and 4.4 pmoles of unlabeled forward primer in a BigDye Terminator Cycle Sequencing Reaction as described by the supplier (Applied Bio-

systems). Samples were analyzed on the ABI 3100, and sequences were determined with Genescan software (Perkin-Elmer).

BRCA1 Q356R Polymorphism. PCR was performed using the forward primer 5′-GGA CTC CCA GCA CAG AAA AA-3′ and reverse primer 5′-TCC CCA TCA TGT GAG TCA TC-3′. The reaction was conducted in a final volume of 15 μl containing 0.5 ng/μl genomic DNA, 0.5 nmol/liter forward primer, 0.5 nmol/liter reverse primer, 0.2 mmol/liter dNTP, 1.5 mmol/liter $MgCl_2$ (Life Technologies, Inc.), 1 × Life Technologies, Inc. PCR buffer (part #Y02028), and 0.025 units/μl TaqDNA polymerase (Life Technologies, Inc.; cat. #10342-020). PCR conditions consisted of an initial denaturing step at 95°C for 3 min, 30 cycles of 94°C for 45 s, 57°C for 45 s, and 72°C for 1 min, an extension step at 72°C for 10 min, then at 4°C until digested. A digest of the amplicon was performed by combining 15 μl of the PCR amplification, 2 μl of 10 × NEB Buffer 4, and 10 units of *Alu*NI (New England Biolabs, Beverly, MA) in a final volume of 20 μl. Samples were incubated at 37°C for 4.5 h and analyzed immediately on a 2% agarose gel. The undigested arginine (R) allele can be seen as a band at 211 bp, whereas the glutamine (Q) allele is represented by the digestion products at 134 and 77 bp.

A fraction of samples were subjected to sequencing to confirm results obtained using the restriction fragment length analysis described above. For sequencing, completed 50-μl PCR amplifications were purified using QIAquick 96 vacuum filter plates (Qiagen) and reconstituted in 150 μl of elution buffer. A sequencing reaction was performed using 1 μl of purified product and 4.4 pmoles of unlabeled forward primer in a BigDye Terminator Cycle Sequencing Reaction as described by the supplier (Applied Biosystems). Samples were analyzed on the ABI 3100, and sequences were determined with Genescan software (Perkin-Elmer).

Statistical Analysis. The genotype data were tested for Hardy-Weinberg Equilibrium using the χ^2 goodness of fit test. ORs and 95% CIs for the association between the BRCA1 and BRCA2 polymorphisms and epithelial ovarian cancer were calculated for all cases, all serous cases, invasive cases, and invasive serous cases. Logistic regression analysis was used to compute adjusted ORs accounting for age, race, and other potential confounders. Crude ORs are reported as well as adjusted ORs from a multivariable logistic regression model, which included race and age and other potential confounders. Menopausal status, tubal ligation, oral contraceptive use, family history of breast or ovarian cancer in first and second degree relatives, and parity were individually tested to determine whether they changed the crude OR by 10%. All calculations were performed with SAS 8.0 (SAS Institute, Inc., Cary, NC) using unconditional logistic regression. With our current sample size of ~300 cases and 400 controls, we have 80% power to detect an OR of ≥1.6 with type 1 error level equal to 0.05 for BRCA2 N372H and BRCA1 P871L polymorphisms. Because of the lower prevalence of the BRCA1 Q356 R allele, we have 80% to detect an OR of 1.9.

RESULTS

The distributions of epidemiological risk factors for the cases and controls are shown in Table 1. Cases and controls are

Table 1 Demographic and pathologic characteristics of cancer cases and controls

	Cases (n = 312) n (%)	Controls (n = 401) n (%)
Age, years		
20–54	155 (50)	210 (52)
55–75	157 (50)	191 (48)
Race		
Caucasian	278 (89)	349 (87)
African-American	34 (11)	52 (13)
Menopause status		
Pre/peri	121 (39)	165 (41)
Post	191 (61)	236 (59)
Ever-smoked 100 cigarettes	147 (47)	201 (50)
OC use (years)		
Nonusers	108 (35)	133 (34)
<2	66 (21)	64 (16)
2–5	59 (19)	81 (20)
>5	76 (25)	118 (29)
Unknown	3	5
Pregnancies resulting in live birth		
0	60 (19)	54 (13)
1	58 (19)	66 (16)
2	106 (34)	147 (37)
3	52 (17)	84 (21)
4+	36 (12)	50 (12)
Had tubal ligation	75 (24)	132 (33)
BMI		
15–23	70 (23)	99 (26)
23–26	69 (22)	95 (24)
26–30	65 (21)	94 (24)
>30	105 (34)	98 (25)
Family history of breast or ovarian cancer in a 1° relative		
Yes	52 (17)	66 (16)
No	260 (83)	355 (84)
Tumor behavior		
Borderline	75 (24)	
Invasive	237 (76)	
Tumor stage[a]		
I/II	132 (43)	
III/IV	178 (57)	
Tumor histology		
Serous	185 (59)	
Endometrioid	38 (12)	
Mucinous	40 (13)	
Adenocarcinoma	22 (7)	
Clear cell	15 (5)	
Other	12 (4)	

[a] Two missing tumor stage. Three cases and 15 controls missing body mass index.

similar in age and race, with 11% of cases and 13% of controls being self-reported as African-American. The distributions of menopausal status, oral contraceptive use, and family history of breast or ovarian cancer are also similar. Controls tended to have had more pregnancies than cases and were more likely to have had tubal ligations (33 *versus* 24%). The distributions of ovarian tumor characteristics, including stage, histology, and tumor behavior, are noted in Table 1. Over half of cases were diagnosed with stage III/IV disease, 76% had invasive cancers, and 59% were of the serous histological subtype.

BRCA2 N372H Polymorphism. Genotyping was performed using automated DNA sequencing in 312 cases and 398
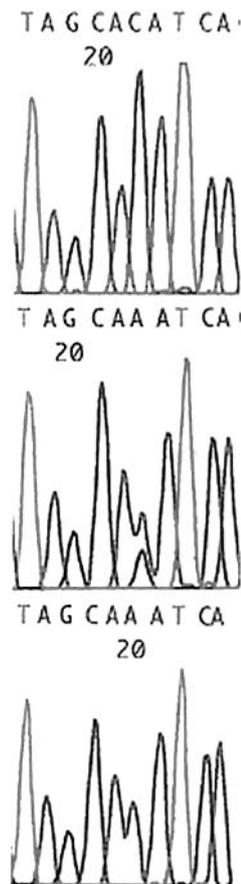
Fig. 1 DNA sequencing of BRCA2 N372H polymorphism. *Top panel,* HH homozygote; *middle panel,* NH heterozygote; *bottom panel,* NN homozygote.

Table 2 ORs and 95% CIs for the association between ovarian cancer risk and BRCA1 and BRCA2 polymorphisms

Genotype	Cases n (%)	Controls n (%)	Adjusted OR[a]	95% CI
BRCA2 N372H				
NN	169 (54)	227 (57)	1.0	(Referent)
NH	128 (41)	146 (37)	1.2	(0.8–1.6)
HH	15 (5)	25 (6)	0.8	(0.4–1.5)
NH/HH	143 (46)	171 (43)	1.1	(0.8–1.5)
BRCA1 P871L				
PP	127 (42)	141 (36)	1.0	(Referent)
PL	123 (40)	174 (45)	0.8	(0.6–1.1)
LL	55 (18)	73 (19)	0.9	(0.6–1.5)
PL/LL	178 (58)	247 (64)	0.8	(0.6–1.1)
BRCA1 Q356R				
QQ	275 (91)	344 (90)	1.0	(Referent)
QR	27 (9)	39 (10)	0.9	(0.5–1.4)
RR	1 (0)	1 (0)	1.3	(0.1–20.2)
QR/RR	28 (9)	40 (10)	0.9	(0.5–1.4)

[a] Adjusted for race and age.

Table 3 ORs and 95% CIs for the association of serous ovarian cancer risk and BRCA1 and BRCA2 polymorphisms

Genotype	Cases n %	Controls n %	Adjusted OR[a]	95% CI
BRCA2 N372H				
NN	100 (54)	227 (57)	1.0	(Referent)
NH	76 (41)	146 (37)	1.2	(0.8–1.7)
HH	9 (5)	25 (6)	0.8	(0.4–1.8)
NH/HH	85 (46)	171 (43)	1.1	(0.8–1.6)
BRCA1 P871L				
PP	60 (39)	141 (36)	1.0	(Referent)
PL	64 (42)	174 (45)	0.9	(0.6–1.3)
LL	30 (19)	73 (19)	1.0	(0.6–1.7)
PL/LL	94 (61)	247 (64)	0.9	(0.6–1.3)
BRCA1 Q356R				
QQ	167 (92)	344 (90)	1.0	(Referent)
QR	14 (8)	39 (10)	0.7	(0.4–1.4)
RR	0 (0)	1 (0)		Inestimable
QR/RR	14 (8)	40 (10)	0.7	(0.4–1.4)

[a] Adjusted for race and age.

controls (Fig. 1). Confirmatory sequencing performed in a random 10% subset of cases and controls showed 97.2% (70 of 72) agreement. Among control women, the distribution of genotypes was found to be in Hardy-Weinberg Equilibrium (χ^2 = 0.055, P = 0.8). The frequency of the rare H allele was 0.246 among all controls and 0.257 among Caucasian controls. There was no significant association between the N372H polymorphism and risk of ovarian cancer using either a recessive or codominant model (Table 2). Overall, 5% of invasive and borderline ovarian cancer cases and 6% of controls were homozygous for the rare H allele (OR = 0.8, 95% CI 0.4–1.5). Similar ORs were seen in the subgroups with invasive cancers (OR = 0.8, 95% CI 0.4–1.7), all serous tumors (OR = 0.8, 95% CI 0.4–1.8; Table 3), and invasive serous cancers (OR = 0.7, 95% CI 0.3–1.9).

BRCA1 P871L Polymorphism. Genotyping was performed in 305 cases and 388 controls using a TaqMan assay (Fig. 2). DNA sequencing was performed in 14 samples, including both homozygotes and heterozygotes, and there was complete concordance with the genotypes obtained using the TaqMan assay. Genotype frequencies differed dramatically between Caucasian and African-American control women. Among 337 Caucasian controls, there were 137 PP (41%), 158 PL (47%),

and 42 LL (12%), whereas among 51 African-Americans, there were 4 PP (8%), 16 PL (31%), and 31 LL (61%). Frequencies of the P and L alleles were 0.64 and 0.36 among Caucasians and 0.24 and 0.76 among African-Americans (P < 0.0001). In both groups of control women, the distribution of genotypes was found to be in Hardy-Weinberg Equilibrium (Caucasians: χ^2 = 0.117, P = 0.7; African-Americans: χ^2 = 0.84, P = 0.36). There was no significant association between the P871L polymorphism and risk of ovarian cancer using a recessive or codominant model in the entire group (Table 2) or in either racial group alone (data not shown). Overall, 18% of invasive and borderline ovarian cancer cases and 19% of controls were homozygous for the L allele (OR = 0.9, 95% CI 0.6–1.5). Similar ORs were seen in the subgroups with invasive cancers (OR = 0.8, 95% CI 0.4–1.3), all serous tumors (OR = 1.0, 95% CI 0.6–1.7; Table 3), and invasive serous cancers (OR = 0.8, 95% CI 0.4–1.5).

BRCA1 Q356R Polymorphism. Genotyping was performed in 303 cases and 384 controls using restriction fragment-
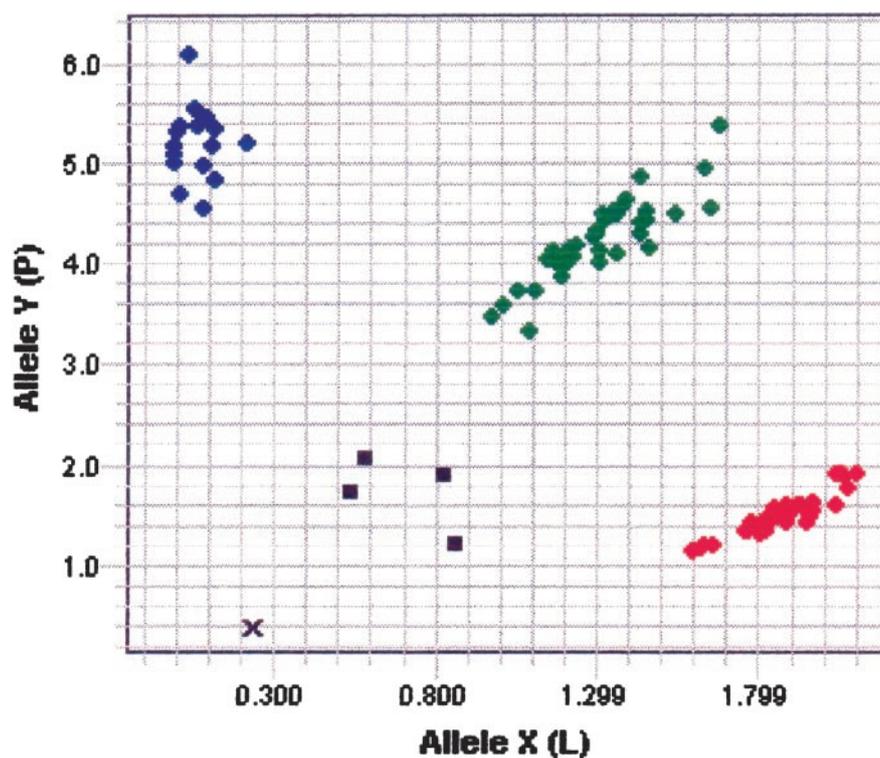
Fig. 2 BRCA1 P871L genotyping using TaqMan assay. PP homozygotes (*blue*), LL homozygotes (*red*), PL heterozygotes (*green*), and water controls (■). The black X represents an uninterpretable outlier.
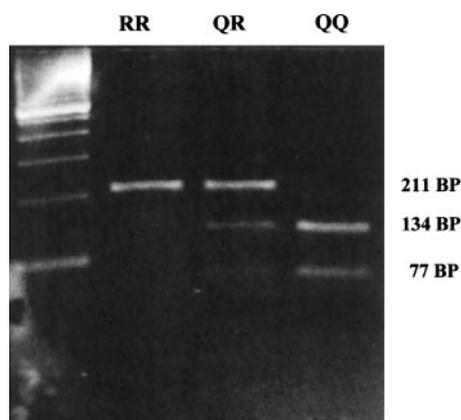

Fig. 3 BRCA1 Q356R genotyping using restriction fragment length analysis with PCR products resolved on a 2% agarose gel. The undigested R allele is represented as a 211-bp band and the Q allele as digest fragments of 134 and 77 bp. *Lane 1,* RR homozygote; *Lane 2,* QR heterozygote; *Lane 3,* QQ homozygote.

length analysis (Fig. 3). The R allele was both rarer and more prone to be incorrectly scored, as a band corresponding to the R allele could represent the misleading remnant of incomplete digestion. In contrast, the Q allele, being a digest product, was unlikely to be present falsely. Confirmatory DNA sequencing was performed in 95 cases, including all 54 samples that were homozygous or heterozygous for the R allele. In only one of 95 samples sequenced was the genotype changed yielding an accuracy of 98.9%. Among control women, the distribution of

genotypes was found to be in Hardy-Weinberg equilibrium (χ^2 = 0.009, P = 0.928). There was no significant association between the Q356R polymorphism and risk of ovarian cancer (Table 2). The rates of heterozygosity and homozygosity for the R allele were 9% and <1% in ovarian cancer cases and 10% and <1% in controls. Using a codominant model, the combined OR was 0.9 (95% CI 0.5–1.4). Similar ORs were seen in the subgroups with invasive cancers (OR = 0.8, 95% CI 0.4–1.4), all serous tumors (OR = 0.7, 95% CI 0.4–1.4; Table 3), and invasive serous cancers (OR = 0.7, 95% CI 0.3–1.4).

None of the BRCA1 or BRCA2 polymorphisms were associated with ovarian cancer risk when cases were stratified by race, age of onset, parity, history of oral contraceptive use, or family history of breast/ovarian cancer (data not shown).

DISCUSSION

Previous reports have examined the relationship between polymorphisms in genes such as the progesterone receptor (17–19), androgen receptor (20, 21), CYP17 (22, 23), p53 (24, 25) and epoxide hydrolase (26, 27), and ovarian cancer risk. Positive associations reported by some groups have not been confirmed by others, and this likely is attributable to methodological weaknesses, including being hospital rather than population based and using controls that are poorly matched with respect to the presence of ovaries, age, and race (28). We have begun to examine candidate susceptibility polymorphisms in the context of a population-based, case control study of newly diagnosed ovarian cancer cases in central and eastern North Carolina.

Common polymorphisms in BRCA1 and 2 are high priority breast/ovarian cancer susceptibility candidates, because germ-

line mutations in these genes strikingly increase risk. In this regard, it was reported by a group in the United Kingdom that homozygosity for the H allele of the N372H polymorphism in exon 10 of BRCA2 conferred a 1.31-fold (95% CI 1.07–1.61) increased risk of breast cancer (10). It was estimated that ~2% of all breast cancers could be attributed to this polymorphism. Subsequently, a second study from Australia reported that homozygosity for the H allele was more common in cases compared with controls (9.2 *versus* 6.5%) and was associated with an elevated risk of breast cancer (OR = 1.46, 95% CI 1.05–2.07; Ref. 11).

The Australian group also examined whether N372H affects ovarian cancer risk (12). This study included 1121 ovarian cancer cases and 2643 controls from British and Australian studies. The HH genotype was associated with an increased risk of ovarian cancer in both studies, and the risk estimate for the pooled studies was 1.36 (95% CI 1.04–1.77, $P = 0.03$). There was a suggestion that this risk may be greater for ovarian cancers of the serous subtype (OR = 1.66, 95% CI 1.17–2.54, $P = 0.005$). Among 480 serous ovarian cancers, 10% were HH homozygotes compared with only 6.5% of 2643 controls.

Unlike previous studies of BRCA2 N372H that used Taq-Man or allele-specific oligonucleotide assays (10–12), ours was the first study that used DNA sequencing to evaluate the genotype of all subjects. In the NCOC study, the frequency of the H allele among Caucasian controls (0.257) is essentially the same as that reported in previous studies (10–12). However, a relationship was not observed between the BRCA2 N372H polymorphism and overall risk of ovarian cancer or of the serous subtype. Although we examined >700 subjects, our study lacked sufficient power to ascertain small increases in risk, particularly in subset analyses, such as among invasive serous cases. The fact that our overall fraction of HH homozygotes was lower in cases than in controls (5 *versus* 6%, OR of 0.8; 95% CI 0.4–1.5), while not precluding the previous finding of an increased risk in cases as reported by Auranen *et al.* (3), it certainly does not lend it support.

The initial report on BRCA2 N372H suggested that there was a deficiency of HH and NN homozygotes among female controls relative to expected Hardy-Weinberg equilibrium (10). In contrast, an excess of homozygotes was observed in male controls. A deficiency of HH homozygotes was noted in newborn girls, whereas in chromosomally normal female abortuses, an excess of HH genotypes were seen. Taken together, these findings are suggestive that the H variant might affect fetal survival in a sex-dependent manner. A slight deficit of HH homozygotes was also seen in female controls in the Australian breast cancer study (50 observed *versus* 53.7 expected; Ref. 11) and ovarian cancer study (172 observed *versus* 190 expected; Ref. 29). In the current study, we found that the distribution of genotypes for BRCA2 N372H in controls conformed closely to Hardy-Weinberg equilibrium (25 HH homozygotes observed compared with 24 expected). Although this differs somewhat from the results of the previous studies discussed above, it is notable that the initial study of N372H included several control populations. A consistent excess of homozygotes was observed in several British populations, but similar to the findings in the NCOC Study, this was not the case in a Finnish population. In the ovarian cancer cases in the Finnish population, there were somewhat more NH heterozygotes than expected, rather than an excess of HH homozygotes.

Although the lifetime risk of breast cancer is similarly high in carriers of either BRCA1 or BRCA2 mutations, ovarian cancer risk is significantly higher in BRCA1 carriers. Thus, functional polymorphisms in BRCA1 might be postulated to be more likely to affect ovarian cancer risk than those in BRCA2. There are 10 polymorphisms in BRCA1 with allele frequencies >5% in Caucasians; however, only five of these (Q356R, P871L, E1038G, K1183R, and S1613G) result in amino acid changes (13). These polymorphisms, with the exception of Q356R, are in significant linkage disequilibrium and generally are inherited as part of a shared haplotype. As a result, only three halpotypes occur with a frequency of >1.3%, and the effect of all of these can be ascertained by analyzing Q356R and P871L (13, 14). Durocher *et al.* (13) examined the allele frequencies of all 10 common BRCA1 polymorphisms in a group of affected women from breast/ovarian cancer families and in control populations from Utah and Quebec. All these polymorphisms were found to be in Hardy-Weinberg equilibrium, and P871L was the only one in which a significant difference in allele frequency was seen between breast/ovarian cancer cases (L = 0.42) and controls (L = 0.28). The authors acknowledged this difference could be attributable to population admixture, because the breast/ovarian cancer cases were ascertained from many different centers. Janezic *et al.* (15) also published preliminary data consistent with an increased risk of ovarian cancer attributable to P871L from a population-based study in California in which BRCA1 sequencing was performed in women with ovarian cancer. They examined the significance of observed BRCA1 polymorphisms in 24 ovarian cancer cases and 24 sister controls. P871L was the only one in which there was a higher frequency of the L allele in cases (0.38) compared with controls (0.29), but this difference was not statistically significant.

Dunning *et al.* (14) examined the P871L polymorphism in three case control studies of breast cancer (572 total controls and 801 total breast cancers) and a hospital-based series of 237 consecutive ovarian cancers in the United Kingdom. There was no relationship between P871L genotype and risk of either breast or ovarian cancer. The frequency of the L allele was 0.32 in controls and 0.33 in ovarian cancer cases. Although no relationship with ovarian cancer risk was seen, their control group was selected to match the breast cancer cases, rather than the ovarian cancer cases. Our study represents the first population-based, case control study of BRCA1 polymorphisms in which controls were specifically matched to ovarian cancer cases with respect to important confounders such as age, race, and the presence of ovaries. We did not find an association between P871L genotype and ovarian cancer risk. As noted above with regard to BRCA2 N372H, the sample size used in our study, although relatively large, is insufficient to exclude a small increased risk attributable to BRCA1 P871L, particularly in important subsets, such as invasive serous ovarian cancers. The frequency of LL homozygotes was slightly lower in cases relative to controls, however; thus, it is unlikely that a significant OR would be achieved with a larger sample size.

African-Americans comprise >10% of our study population and have a lower incidence of ovarian cancer (ages 40–59: 17/100,000; ages ≥ 60: 24.5/100,000) relative to Caucasians

(ages 40–59: 26/100,000; ages ≥ 60: 38.4/100,000; Ref. 30). One possible explanation for the racial difference in ovarian cancer incidence may be differences in frequencies of susceptibility alleles. In this regard, we observed a striking racial difference in BRCA1 P871 allele frequencies. In Caucasians, the P allele was more common (0.64), whereas in African-Americans, the L allele predominates (0.76). This polymorphism was not associated with ovarian cancer risk in either race, however.

Previous studies of BRCA1 polymorphisms have been performed predominantly in Caucasians, however, as demonstrated above; allele frequencies may vary considerably between races. Racial variation in allele frequencies of P871L probably explains the higher L allele frequency in ovarian cancer cases relative to controls in the Durocher *et al.* study (13). Ovarian and breast cancer cases were from collected series of high-risk families, some of which likely were African-American, whereas control subjects were from geographic areas (Utah, Quebec) where few African-Americans reside. In view of the high frequency of the L allele in the African-American population, a slightly higher fraction of African-Americans among cases relative to controls would skew the distribution of allele frequencies between the groups. Racial difference between cases and controls cannot account for the association between the L allele and ovarian cancer in the Janizec study, because controls were sisters of the cases (15). However, this analysis was much too small to allow for meaningful conclusions, because it was based on only 24 cases.

The BRCA1 Q356R polymorphism segregates independently from the other common BRCA1 polymorphisms. In our large population-based, case control study, we did not find evidence to support a relationship between Q356R and ovarian cancer risk. Likewise, in the above noted study of BRCA1 polymorphisms by Durocher *et al.* (13) the rare R allele was not associated with cancer risk and had allele frequencies of ~6% in breast cancer cases, ovarian cancer cases, and controls. Dunning reported that the R allele was slightly more common among controls (7%) than breast cancer cases (6%), and RR homozygotes were only found among controls (14). Because of the rarity of the R allele, ORs were reported using a codominant model combining RR homozygotes and QR heterozygotes. The OR for breast cancer was 0.88 (95% CI 0.63–1.23), suggesting that the rare R allele might be protective against breast cancer. In examining 230 ovarian cancer cases, no relationship was seen between Q356R genotype and ovarian cancer risk. Janezic *et al.* (15) found that women in California with a family history of ovarian cancer had a higher frequency of the R allele compared with women with ovarian cancer lacking a family history. This was interpreted as consistent with an association of the R allele with ovarian cancer risk. Conversely, Smith *et al.* (16) reported that among women with ovarian cancer, the R allele of the Q356R polymorphism was more common in those who lacked a family history of cancer.

Although we did not observe associations between polymorphisms in BRCA1 or BRCA2 and ovarian cancer risk, it is possible that these polymorphisms might affect risk via gene–gene or gene–environment interactions, *e.g.,* the penetrance of deleterious BRCA mutations could be affected by polymorphisms in these same genes. Alternatively, these polymorphisms might alter risk exclusively in the setting of exposures, such as high lifetime ovulatory cycles or oral contraceptive use. We are continuing accrual in the NCOC Study and in the future hope to have sufficient power, either alone or in collaboration with other groups, to examine gene–gene and gene–environment interactions.

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