Null Results in Brief

Missense Polymorphisms in *BRCA1* and *BRCA2* and Risk of Breast and Ovarian Cancer

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Abstract

Purpose: **BRCA1 and BRCA2 are key tumor suppressors with a role in cellular DNA repair, genomic stability, and checkpoint control. Mutations in *BRCA1* and *BRCA2* often cause hereditary breast and ovarian cancer; however, missense polymorphisms in these genes pose a problem in genetic counseling, as their impact on risk of breast and ovarian cancer is unclear.**
Experimental Design: **We resequenced *BRCA1* and *BRCA2* in 194 women with a familial history of breast and/or ovarian cancer and identified nine possibly biologically relevant polymorphisms (*BRCA1 Gln356Arg, Pro871Leu, Glu1038Gly, Ser1613Gly,* and *Met1652Ile*. *BRCA2 Asn289His, Asn372His, Asp1420Tyr,* and *Tyr1915Met*). We evaluated risk of breast and/or ovarian cancer by these polymorphisms in a prospective study of 5,743 women from the general population followed for 39 years and in a case-control study of 1,201 breast cancer cases and 4,120 controls.**
Results: **We found no association between heterozygosity or homozygosity for any of the nine polymorphisms and risk of breast and/or ovarian cancer in either study. We had 80% power to exclude hazard/odds ratios for heterozygotes and/or homozygotes for all nine missense polymorphisms above 1.3 to 3.3 in the prospective study, and above 1.2 to 3.2 in the case-control study.**
Conclusions: **Heterozygosity and homozygosity of any of the examined nine *BRCA1* and *BRCA2* missense polymorphisms cannot explain the increased risk of breast and/or ovarian cancer observed in families with hereditary breast and/or ovarian cancer. Therefore, genetic counseling of such families safely can disregard findings of these missense polymorphisms.** (Cancer Epidemiol Biomarkers Prev 2009;18(8):2339–42)

Introduction

Rare mutations [minor allele frequency (MAF), <1%] in *BRCA1* and *BRCA2* cause 5% to 10% of all breast and ovarian cancer and 20% to 30% of hereditary breast and ovarian cancer cases (1). During screenings of breast and ovarian cancer cases with a strong familial history of breast and/or ovarian cancer, missense polymorphisms (MAF, ≥1%) are often discovered. These polymorphisms pose a problem in genetic counseling, as an impact on risk of breast and/or ovarian cancer has not been excluded convincingly.

We resequenced *BRCA1* and *BRCA2* in 194 women with a familial history of breast and/or ovarian cancer and identified nine nonconservative amino acid substituting and thus possibly biologically relevant polymorphisms (*BRCA1 Gln356Arg, Pro871Leu, Glu1038Gly, Ser1613Gly,* and *Met1652Ile. BRCA2 Asn289His, Asn372His, Asp1420Tyr,* and *Tyr1915Met*). Several of the chosen polymorphisms were furthermore located in regions of BRCA1 and BRCA2 known to be interaction sites for key partner proteins (2, 3), and many of the amino acids concerned are conserved in human, mouse, and rat. We evaluated risk of breast and/or ovarian cancer associated with these polymorphisms in a prospective study of 5,743 women from the general population followed for 39 years, and in a case-control study of 1,201 breast cancer cases and 4,120 controls.

Materials and Methods

For a full description of methods, refer to Dombernowsky et al. (4) and Weischer et al. (5).

Participants. First, we resequenced *BRCA1* and *BRCA2* in 194 Danish women referred for genetic counseling due to a familial history of breast and/or ovarian cancer.

Second, we did a prospective population-based study of 5,743 White women from the Danish general population participating in the Copenhagen City Heart Study (4-6). Diagnoses of invasive cancer (WHO International Classification of Diseases 7th edition) for the whole cohort from 1947 through August 11th 2007 were obtained from the Danish Patient Registry and the Danish Cancer Registry. Follow-up was 100% complete.

Received 5/11/09; revised 5/27/09; accepted 6/4/09; published online 8/6/09.
Grant support: Danish Medical Research Council, The Copenhagen County Foundation, and The Danish Heart Foundation.
Statement of originality: This is an original report that has not been published previously.
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doi:10.1158/1055-9965.EPI-09-0447

Table 1. Incidence and risk of breast and/or ovarian cancer in the prospective study according to genotype

	Participants (*n*)	Breast and/or ovarian cancers (*n*)	Incidence (95% CI; per 10,000 person-year)	Log-rank *P*	Hazard ratio (95% CI)		80% power hazard ratio*
					Age adjustment	Multifactorial adjustment	
BRCA1							
Gln356Arg							
Gln-Gln	4,976	356	21 (19-23)		1.0	1.0	
Gln-Arg	686	60	25 (20-33)	0.06	1.3 (1.0-1.7)	1.1 (0.9-1.5)	1.4
Arg-Arg	21	3	39 (13-121)	0.07	2.7 (0.9-8.5)	2.2 (0.7-6.9)	3.3
Pro871Leu							
Pro-Pro	2,443	183	22 (19-25)		1.0	1.0	
Pro-Leu	2,582	189	21 (19-25)	0.91	1.0 (0.8-1.2)	1.0 (0.8-1.2)	1.3
Leu-Leu	657	47	21 (16-28)	0.92	1.0 (0.7-1.4)	1.0 (0.7-1.4)	1.5
Glu1038Gly							
Glu-Glu	2,578	194	22 (19-25)		1.0	1.0	
Glu-Gly	2,498	183	21 (19-25)	0.89	1.0 (0.8-1.2)	1.0 (0.8-1.2)	1.3
Gly-Gly	607	42	20 (15-28)	0.75	0.9 (0.7-1.3)	0.9 (0.7-1.3)	1.5
Ser1613Gly							
Ser-Ser	2,571	193	22 (19-25)		1.0	1.0	
Ser-Gly	2,498	182	21 (18-25)	0.84	1.0 (0.8-1.2)	1.0 (0.8-1.2)	1.3
Gly-Gly	614	44	21 (16-28)	0.89	1.0 (0.7-1.4)	1.0 (0.7-1.3)	1.5
Met1652Ile							
Met-Met	5,547	407	21 (19-24)		1.0	1.0	
Met-Ile + Ile-Ile†	137	12	26 (15-46)	0.45	1.3 (0.7-2.2)	1.3 (0.7-2.4)	1.9
BRCA2							
Asn289His							
Asn-Asn	5,351	390	21 (19-24)		1.0	1.0	
Asn-His + His-His†	333	29	25 (18-36)	0.64	1.1 (0.7-1.6)	1.1 (0.8-1.6)	1.6
Asn372His							
Asn-Asn	2,921	207	21 (18-24)		1.0	1.0	
Asn-His	2,322	179	23 (19-26)	0.78	1.0 (0.8-1.3)	1.0 (0.9-1.3)	1.3
His-His	440	33	22 (15-30)	0.75	1.1 (0.7-1.5)	0.9 (0.6-1.4)	1.6
Asp1420Tyr							
Asp-Asp	5,542	410	22 (20-24)		1.0	1.0	
Asp-Tyr + Tyr-Tyr†	142	9	19 (10-36)	0.81	0.9 (0.5-1.8)	0.8 (0.4-1.7)	1.9
Tyr1915Met							
Tyr-Tyr	5,317	396	22 (20-24)		1.0	1.0	
Tyr-Met + Met-Met†	367	23	19 (12-28)	0.69	0.9 (0.6-1.4)	0.9 (0.6-1.4)	1.6

NOTE: Multifactorial adjustment included age (<50 y vs ≥50 y), body mass index (BMI ≤25 kg/m^2 vs 25 kg/m^2 < BMI ≤30 kg/m^2 vs BMI >30 kg/m^2), weekly alcohol intake (0 grams/wk vs 1-168 grams/wk vs >168 grams/wk), parity (number of children), nulliparity (yes/no), use of oral contraceptive drugs at the time of examination (yes/no), menopausal status (premenopausal/postmenopausal), and use of hormonal replacement therapy at the time of examination (yes/no).
Abbreviation: CI, confidence interval.
*We had 80% power at two-sided *P* value of <0.05 to exclude the hazard ratios given.
†Homozygotes were pooled with heterozygotes in analyses of polymorphisms with a MAF of <5%.

Third, we did a case-control study that included 1,201 White women with invasive breast cancer consecutively recruited at Herlev Hospital, Copenhagen University Hospital, between January 2002 and August 2004 (5, 7). Controls were 4,120 White women from the general population (The Copenhagen City Heart Study) within the same age range as the patients, who had no history of breast cancer before 11th of August 2007.

Genotyping. Genotyping of prospective and case-control study participants was done using TaqMan assays (Applied Biosystems).

Statistical Analyses. In the prospective study, we used log-rank test and Cox regression with delayed entry and age as the underlying time variable. In the case-control study, one breast cancer case was matched with up to four controls within 1-y age strata and conditional logistic regression was done. To increase power, homozygotes were pooled with heterozygotes in analyses of polymorphisms with a MAF of <5%.

Results

We achieved call rates between 99.9% and 100% for all genotypes in both studies. Characteristics of prospective and case-control study participants at study entry were as previously described (4, 5).

In the general population, minor allele frequencies were *BRCA1 Gln356Arg* 6.4%, *BRCA1 Pro871Leu* 34.3%, *BRCA1 Glu1038Gly* 32.7%, *BRCA1 Ser1613Gly* 32.8%, *BRCA1 Met1652Ile* 1.2%, *BRCA2 Asn289His* 3.0%, *BRCA2 Asn372His* 28.2%, *BRCA2 Asp1420Tyr* 1.3%, and *BRCA2 Tyr1915Met* 3.3%. All missense polymorphisms were in Hardy-Weinberg equilibrium (χ^2 test: $P = 0.61$, $P = 0.52$, $P = 0.96$, $P = 0.84$, $P = 0.21$, $P = 0.38$, $P = 0.47$, $P = 0.91$, and $P = 0.96$, respectively). *BRCA1 Pro871Leu, Glu1038Gly,* and *Ser1613Gly* were in strong linkage disequilibrium (D' = 0.99-1.00; r^2 = 0.91-0.99; data not shown).

In the prospective study, 419 women were diagnosed with breast and/or ovarian cancer during

39 years of follow-up. Of these, 342 had breast cancer, 71 had ovarian cancer, and 6 had both breast and ovarian cancer. We found no association between heterozygosity or homozygosity for any of the nine *BRCA1* and *BRCA2* polymorphisms and risk of breast and/or ovarian cancer in the prospective study (Table 1). Table 1 also shows the hazard ratios we had 80% power to exclude. Furthermore, heterozygosity or homozygosity of any combination of two of the nine polymorphisms did not associate with increased risk of breast and/or ovarian cancer (data not shown). After Bonferroni correction, there were no significant interactions between either of the nine polymorphisms and either of the eight covariates (see Table 1 legend) on risk of breast and/or ovarian cancer [with 14 genotypes tested, the new level of significance was $P = 0.05/(8 \times 14) = 0.00045$].

Likewise, we found no association between heterozygosity or homozygosity of any of the nine missense polymorphisms and risk of breast cancer in the case-control study (Table 2). Table 2 furthermore shows the odds ratios we had 80% power to exclude. After Bonferroni correction, there were no significant interactions between either of the nine polymorphisms and either of the eight covariates (see Table 2 legend) on risk of breast cancer (new level of significance $P = 0.00045$).

Discussion

Resequencing of *BRCA1* and *BRCA2* is offered as part of genetic counseling to women with a strong familial history of breast and/or ovarian cancer. On average, such screenings identify a causative mutation in 20% to 30% of patients (1). However, we identified amino acid substituting polymorphisms in 86% of the 194 women who underwent *BRCA1* and *BRCA2* resequencing at Herlev Hospital. Clinical geneticists generally regard these common polymorphisms as unimportant, although the literature to support this has been scarce and contradictive (8-24). Importantly, none of the examined nine *BRCA1* and *BRCA2* missense polymorphisms alone or in combination were associated with increased risk of breast and/or ovarian cancer in the general population, or in the breast cancer case-control study.

Previously, some of these missense polymorphisms have been examined in a number of smaller studies. Some of these found associations to breast and/or ovarian cancer (8, 13, 15, 16, 18, 21-23), whereas others did not (9-12, 14, 15, 17, 19, 20, 24). To the best of our knowledge, this is the first large population-based study of these nine missense polymorphisms. As we were not able to detect any associations despite sufficient power, we accordingly conclude that heterozygosity or homozygosity of any of

Table 2. Risk of breast cancer in the case-control study according to genotype

	Cases (*n*)	Controls (*n*)	Odds ratio (95% CI)		80% power odds ratio*
			Age adjustment	Multifactorial adjustment	
BRCA1					
Gln356Arg					
Gln-Gln	1,048	3,589	1.0	1.0	
Gln-Arg	147	513	1.0 (0.8-1.2)	1.0 (0.8-1.3)	1.3
Arg-Arg	5	17	0.9 (0.3-2.4)	0.8 (0.3-2.5)	3.2
Pro871Leu					
Pro-Pro	550	1,756	1.0	1.0	
Pro-Leu	496	1,896	0.8 (0.7-1.0)	0.9 (0.7-1.0)	1.2
Leu-Leu	155	467	1.1 (0.9-1.3)	1.0 (0.8-1.3)	1.4
Glu1038Gly					
Glu-Glu	563	1,854	1.0	1.0	
Glu-Gly	491	1,835	0.9 (0.8-1.0)	0.9 (0.8-1.1)	1.2
Gly-Gly	145	431	1.1 (0.9-1.4)	1.1 (0.8-1.4)	1.4
Ser1613Gly					
Ser-Ser	557	1,850	1.0	1.0	
Ser-Gly	508	1,834	0.9 (0.8-1.1)	1.0 (0.8-1.1)	1.2
Gly-Gly	133	435	1.0 (0.8-1.3)	1.0 (0.7-1.2)	1.4
Met1652Ile					
Met-Met	1,162	4,030	1.0	1.0	
Met-Ile + Ile-Ile†	38	90	1.5 (1.0-2.2)	1.5 (0.9-2.3)	1.8
BRCA2					
Asn289His					
Asn-Asn	1,123	3,878	1.0	1.0	
Asn-His + His-His†	75	242	1.1 (0.8-1.4)	1.1 (0.8-1.5)	1.5
Asn372His					
Asn-Asn	604	2,129	1.0	1.0	
Asn-His	503	1,677	1.1 (0.9-1.2)	1.0 (0.9-1.2)	1.2
His-His	93	313	1.0 (0.8-1.3)	1.0 (0.8-1.3)	1.4
Asp1420Tyr					
Asp-Asp	1,178	4,014	1.0	1.0	
Asp-Tyr + Tyr-Tyr†	22	106	0.7 (0.4-1.1)	0.7 (0.4-1.2)	1.7
Tyr1915Met					
Tyr-Tyr	1,143	3,844	1.0	1.0	
Tyr-Met + Met-Met†	57	276	0.7 (0.5-0.9)	0.7 (0.5-1.0)	1.4

NOTE: Multifactorial adjustment included age (<50 y vs ≥50 y), BMI (≤25 kg/m^2 vs 25 kg/m^2 <BMI ≤30 kg/m^2 vs BMI >30 kg/m^2), weekly alcohol intake (0 grams/wk vs 1-168 grams/wk vs >168 g/wk), parity (number of children), nulliparity (yes/no), use of oral contraceptive drugs at the time of examination (yes/no), menopausal status (premenopausal/postmenopausal), and use of hormonal replacement therapy at the time of examination (yes/no).
*We had 80% power at two-sided *P* value of <0.05 to exclude the odds ratios given.
†Homozygotes were pooled with heterozygotes in analyses of polymorphisms with a MAF of <5%.

the examined nine *BRCA1* and *BRCA2* polymorphisms, alone or in combination, cannot explain the increased risk of breast and/or ovarian cancer observed in families with hereditary breast and/or ovarian cancer. Therefore, genetic counseling of such families safely can disregard findings of these missense polymorphisms.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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Cancer Epidemiology, Biomarkers & Prevention

Correction: Missense Polymorphisms in BRCA1 and BRCA2 and Risk of Breast and Ovarian Cancer

In this article (1), which was published in the August 2009 issue of *Cancer Epidemiology, Biomarkers & Prevention*, the authors reported risk of breast and ovarian cancer by BRCA2 Tyr1915Met. The BRCA2 variant Tyr1915Met should not be "Tyr" but Thr1915Met.

Reference

1. Dombernowsky SL, Weischer M, Freiberg JJ, Bojesen SE, Tybjaerg-Hansen A, Nordestgaard BG. Missense polymorphisms in BRCA1 and BRCA2 and risk of breast and ovarian cancer. Cancer Epidemiol Biomarkers Prev 2009;18:2339–42.

Published online 06/08/2010.

doi: 10.1158/1055-9965.EPI-10-0410

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Cancer Epidemiol Biomarkers Prev 2009;18:2339-2342.