Modification of *BRCA1-* and *BRCA2-*associated Breast Cancer Risk by *AIB1* Genotype and Reproductive History[1]

Timothy R. Rebbeck,[2] Yiting Wang, Philip W. Kantoff, Krishna Krithivas, Susan L. Neuhausen, Andrew K. Godwin, Mary B. Daly, Steven A. Narod, Jean-Sebastian Brunet, Daniel Vesprini, Judy E. Garber, Henry T. Lynch, Barbara L. Weber, and Myles Brown

Departments of Biostatistics and Epidemiology [T. R. R., Y. W.] and Medicine and Genetics [B. L. W.], University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104; Department of Adult Oncology [P. W. K., K. K., J. E. G., M. B.] and Lank Center for Genitourinary Cancer [P. W. K., K. K.], Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02215; Department of Genetic Epidemiology, University of Utah, Salt Lake City, Utah 84108 [S. L. N.]; Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111 [A. K. G., M. B. D.]; Women's College Hospital, Toronto, Ontario M5G 1N8, Canada [S. A. N., J-S. B., D. V.]; and Department of Preventive Medicine, Creighton University, Omaha, Nebraska 68179 [H. T. L.]

ABSTRACT

Women who have inherited a germ-line mutation in the *BRCA1* or *BRCA2* (*BRCA1/2*) genes have a greatly increased risk of developing breast cancer compared with the general population. However, there is also substantial interindividual variability in the occurrence of breast cancer among *BRCA1/2* mutation carriers. We hypothesize that genes involved in endocrine signaling may modify the *BRCA1/2*-associated age-specific breast cancer penetrance. We studied the effect of alleles at the *AIB1* gene using a matched case-control sample of 448 women with germ-line *BRCA1/2* mutations. We found that these women were at significantly higher breast cancer risk if they carried alleles with at least 28 or 29 polyglutamine repeats at *AIB1*, compared with women who carried alleles with fewer polyglutamine repeats [odds ratio (OR), 1.59; 95% confidence interval (CI), 1.03–2.47 and OR, 2.85; 95% CI, 1.64–4.96, respectively]. Late age at first live birth and nulliparity have been associated with increased breast cancer risk. We observed increases in *BRCA1/2*-associated breast cancer risk in women who were either nulliparous or had their first live birth after age 30 (OR, 3.06; 95% CI, 1.52–6.16). Women were at significantly increased risk if they were nulliparous or had a late age at first live birth and had *AIB1* alleles no shorter than 28 or 29 or more *AIB1* polyglutamine repeats (OR, 4.62; 95% CI, 2.02–10.56 and OR, 6.97; 95% CI, 1.71–28.43, respectively) than women with none of these risk factors. Our results support the hypothesis that pathways involving endocrine signaling, as measured through *AIB1* genotype and reproductive history, may have a substantial effect on *BRCA1/2*-associated breast cancer risk.

INTRODUCTION

Inheritance of a germ-line mutation in the *BRCA1/2*[3] genes is associated with an increased risk of developing breast cancer. However, there is also substantial variability in the penetrance of breast cancer in *BRCA/2* mutation carriers (1–3). These observations imply that germ-line mutations in *BRCA1/2* may be necessary to explain the

Mendelian pattern of cancer in some families, but may not be sufficient to completely describe the interindividual variability in the age-specific risk of cancer. The ability to effectively apply risk prediction or cancer prevention strategies in *BRCA1/2* carriers may therefore depend on the knowledge of risk-modifying factors in addition to *BRCA1/2* mutation status.

There is substantial evidence that *BRCA1/2*-associated breast carcinogenesis is affected by steroid hormones. Epidemiological studies have reported that the penetrance of *BRCA1-* and/or *BRCA2*-associated breast cancer risk is associated with hormone-related exposures, including reproductive history (2, 4). Ablation of ovarian hormone exposure after bilateral prophylactic oophorectomy significantly decreases *BRCA1*-associated breast cancer risk (5). Genotypes involved in steroid hormone metabolism pathways, including the CAG repeat polymorphism found in exon 1 of the *AR* gene (5) modifies *BRCA1*-associated breast cancer penetrance. *BRCA1* has also been shown to be a coactivator of the AR (6), and this activation may be mediated through the effects of p160 coactivators including *SRC-1a, GRIP1,* and *AIB1* (7). *AIB1* Online Mendelian Inheritance in Man (OMIM) accession no. 601937) is a member of the p160 family of transcriptional coactivators that interacts with steroid hormone receptors to enhance ligand-dependent transcription and is required for female reproductive function and mammary gland development (8). *AIB1* was identified in a search for genes that are amplified in breast tumors (9, 10). Anzick *et al.* (10) determined that *AIB1* was amplified in 10% and overexpressed in 64% of a series of 105 breast tumors. Bautista *et al.* (11) subsequently reported that *AIB1* was amplified in 4.8% of another set of breast tumors. Those authors also reported that *AIB1* enhanced estrogen-dependent transcription, suggesting that altered expression of *AIB1* may influence the progression of steroid hormone-dependent cancers.

A glutamine-rich region exists in *AIB1* between residues 1053 and 1123 that is encoded by a CAG_n repeat polymorphism (12). The analogous region of SRC1 interacts directly with the AR and is required for enhancement of AR signaling (13). Therefore, the CAG_n repeat polymorphism in *AIB1* is likely to have a functional effect on steroid hormone signaling pathways. In contrast to *BRCA1/2*-associated breast cancer risk, studies to date of non-*BRCA1/2*-associated breast cancer risk have not found a relationship between germ-line *AR* variants (14) or specific *AIB1* alleles in postmenopausal women (15). However, the results of Park *et al.* (6) and Irvine *et al.* (7) suggest that *AR* and possibly *AIB1* may not play a role in breast carcinogenesis in the presence of an intact and fully functional BRCA1 protein. To determine whether allelic variation in genes governing hormonal signaling may be involved in modification of *BRCA1/2*-associated cancer risk, we evaluated whether germ-line variation in *AIB1* was associated with the penetrance of *BRCA1/2*-associated breast cancers. We report that the polyglutamine repeat polymorphism in *AIB1* is significantly associated with breast cancer risk in women who carry a

disease-associated germ-line mutation in *BRCA1/2*, and that this risk modification is associated with variation in reproductive history.

MATERIALS AND METHODS

Study Participants. A cohort of 656 women who inherited germ-line *BRCA1/2* mutations was ascertained through families with a history of breast and/or ovarian cancer at Creighton University (Omaha, NE), the Dana-Farber Cancer Institute (Boston, MA), the University of Michigan (Ann Arbor, MI), Fox Chase Cancer Center (Philadelphia, PA), the University of Pennsylvania (Philadelphia, PA), the University of Utah (Salt Lake City, UT), or Women's College Hospital (Toronto, Canada). These women were self- or physician-referred to risk evaluation clinics or hereditary breast cancer research studies because of a strong family history of breast and/or ovarian cancer and provided written informed consent for research under protocols approved by the institutional review boards at each institution. Of these women, 330 (50.3%) have been diagnosed with breast cancer.

To minimize the potential for biases using a retrospectively ascertained cohort, a nested case-control sample was generated using an incidence density sampling design. Women were included as breast cancer cases if they had developed an invasive breast cancer of any stage or grade. Women were excluded as cases if they had undergone prophylactic mastectomy or oophorectomy before the date of their breast cancer diagnosis. In addition, women were excluded as cases if they had a diagnosis of ovarian cancer before the date of their breast cancer diagnosis, because these women may have undergone treatments (*e.g.,* oophorectomy) that may have changed their breast cancer risk. Control women were frequency-matched to cases on year of birth (± 5 years), age, and mutation status (*BRCA1* or *BRCA2* mutation). Controls were excluded if they had ever undergone a prophylactic mastectomy or oophorectomy. The resulting case control sample of 448 women consisted of 278 breast cancer cases and 170 controls. The mean age of breast cancer diagnosis in cases was 39.7 years (range, 22–74 years) and the mean age of controls was 41.1 years (range, 19–71 years)

Genotype Analysis. Genotype analysis involved PCR amplification of a region of the *AIB1* coding region beginning at residue 3930 and containing a track of 20–29 trinucleotide repeat alleles that encode polyglutamine residues. The repeat allele sequence studied was $(CAG)_6$ CAA $(CAG)_9$ $(CAA\ CAG)_4$ CAG CAA $(CAG)_2$ CAA for the 29 allele, with decreases in the number of these repeats corresponding to the remaining repeat allele lengths. This is the same repeat polymorphism as that reported by Shirazi *et al.* (16) and Hayashi *et al.* (12). However, the scoring system used here includes all glutamine-coding residues (CAA and CAG), whereas Shirazi *et al.* (16) considered only CAG repeats in their scoring nomenclature.

The PCR amplification protocol consisted of 2.2 μl of $10\times$ buffer (Qiagen), 4 μl of 10 mM deoxynucleotide triphosphates, (Pharmacia Biotech), 1.7 μl each of 10 μM primers (the forward primer labeled with HEX or FAM), 1 μl of Q solution (Qiagen), 0.3 μl of Taq polymerase, 2 μl of 20 ng/μl template DNA, and 9.0 μl of double-distilled water. The primers used for amplification were: forward, 5′-AGT CAC ATT AGG AGG TGG GC-3′; and reverse, 5′-TTC CGA CAA CAG AGG GTG G-3′. The PCR protocol involved a denaturation step at 94°C for 2 min and then 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, with a final elongation cycle of 72°C for 8 min. The amplified PCR products were analyzed on an ABI377 system using a 6% denaturing polyacrylamide gel. Alleles were sized by Genescan 3.0 software (Applied Biosystems). Several samples were sequenced to establish a key that converted PCR fragment length into polyglutamine repeat allele size.

Genotypes were categorized for analysis by allele size and frequency. As with other polyglutamine repeat length alleles (*e.g.,* AR), the size of the repeat length may correspond with function (17, 18). Whereas the functional significance of alleles at *AIB1* is not known, we hypothesize that repeat length may also have functional significance. To evaluate the effect of *AIB1* polyglutamine repeat length on breast cancer risk, we divided the sample into two groups on the basis of the observed distribution of polyglutamine repeats in the sample. First, we compared women who had only alleles with 28 or more repeats compared with those who had one or more alleles of 27 repeats or fewer. Second, we compared women who had only alleles with 29 or more repeats with those who had one or more alleles of 28 repeats or fewer.

Statistical Methods. Unconditional logistic regression analysis was used to estimate the risk of breast cancer by *AIB1* genotype and reproductive factors.

Analyses were undertaken with adjustment for age, year of birth, and reproductive risk factors. These included age at menarche, total number of full term pregnancies (parity), age at first live birth. In addition, we considered a combined reproductive history class comparing women with an early age at first live birth *versus* women who were nulliparous or had a late age at first live birth to simultaneously consider the effects of parity and age at first live birth on breast cancer risk. Interactions between *AIB* genotype and other factors were modeled by stratifying across four categorical levels obtained from dichotomizing genotype (as defined above) and reproductive history (*e.g.,* parous or nulliparous), using a single reference category as a baseline comparison group. Adjustment for age, year of birth, and reproductive history was also undertaken in the stratified and interaction analyses, although reproductive history variables involved in the stratification or interaction were not considered as a confounder in these analyses. Because we frequency-matched controls to cases on age, controls were nonsignificantly older than cases (41.1 *versus* 39.7 years; Kruskal-Wallis $\chi^2 = 1.9$; $df = 1$; $P = 0.168$). Although this insured that controls were at least as old as the diagnosis age of cases, all analyses were undertaken controlling for age to adjust for potential residual age effects. A score test for linear trend of the log odds (19) was used to evaluate whether there was a significant interaction trend of *AIB1* and reproductive factors.

RESULTS

The *AIB1* allele and genotype distributions are presented in Table 1. Because some individuals in this sample are related to one another, genotype frequencies should not be interpreted as reflecting population frequencies for this polymorphism. The most commonly observed alleles were the 26-, 28-, and 29-repeat alleles. Similarly, the 28/28, 28/29, and 29/29 genotypes were most commonly observed, with genotypes containing shorter (less than 26) or longer (more than 29) repeat lengths less common. The frequencies of the 26, 28, and 29 alleles in this sample were 14.0%, 38.2%, and 46.5%, respectively. The frequency of each of the other alleles was <1%.

AIB1 genotype was compared between 278 breast cancer cases and 170 matched controls. As presented in Table 2, women who carried at least one *AIB1* allele of 28 or 29 or more repeats were significantly more likely to have breast cancer than women carrying shorter alleles (OR, 1.59; 95% CI, 1.03–2.47 and OR, 2.85; 95% CI, 1.64–4.96, respectively). Among cases, breast cancer was diagnosed at a mean age of 40.2 $\pm$ 9.0 years among carriers of at least one allele with 28 or more repeats and 38.4 $\pm$ 9.8 years among women with only shorter repeat alleles (Kruskal-Wallis $\chi^2 = 2.17$; $df = 1$; $P = 0.140$). Breast cancer was diagnosed at a mean age of 39.6 $\pm$ 8.2 years among carriers of at least one allele with 29 or more repeats and 39.8 $\pm$ 9.6

Table 1 *Genotype distributions at the polyglutamine repeat polymorphism in* AIB1 *in 448 women who carry germ-line* BRCA1 *or* BRCA2 *mutations*

Polyglutamine repeat length	Cases (%)	Controls (%)
20/28	1 (0.4%)	0 (0%)
22/28	2 (0.7%)	0 (0%)
22/29	0 (0%)	1 (0.6%)
25/26	2 (0.7%)	0 (0%)
26/26	6 (2.2%)	3 (1.8%)
26/28	25 (9.0%)	20 (11.8%)
26/29	29 (10.4%)	29 (17.1%)
26/30	0 (0%)	1 (0.6%)
26/31	0 (0%)	1 (0.6%)
27/29	1 (0.4%)	0 (0%)
---Cutpoint <28[a]		
28/28	32 (11.5%)	27 (15.9%)
28/29	107 (38.5%)	67 (39.4%)
28/30	1 (0.4%)	0 (0%)
28/31	0 (0%)	1 (0.6%)
---Cutpoint <29[a]		
29/29	71 (25.5%)	20 (11.8%)
29/30	1 (0.4%)	0 (0%)
Total	278	170

[a] Denotes cutpoints used in the analyses presented in Tables 2 and 3.

Table 2 *Results of multivariate association analyses for* AIB1 *and other risk factors*

Factor	Cases	Controls	OR (95% CI)[a]
AIB1 <28[b]	66	55	1.0[c]
AIB1 ≥28[b]	212	115	1.59 (1.03–2.47)
AIB1 <29[b]	206	150	1.0[c]
AIB1 ≥29[b]	72	20	2.85 (1.64–4.96)
Menarche <13	128	67	1.0[c]
Menarche ≥13	150	103	0.82 (0.55–1.23)
Parous	241	156	1.0[c]
Nulliparous	37	14	0.67 (0.27–1.67)
Age at first live birth <30	193	145	1.0[c]
Age at first live birth ≥30 or nulliparous	85	25	3.06 (1.52–6.16)

[a] Adjusted for age, year of birth, age at first live birth, age at menarche, parity, or smoking.

[b] Genotype <28, all genotypes containing at least one allele with 27 or fewer polyglutamine repeats; genotype <29, all genotypes containing at least one allele with 28 or fewer polyglutamine repeats; genotype ≥28 or ≥29, genotypes with no allele of 27 or fewer repeats or 28 or fewer repeats, respectively.

[c] Reference group.

years among women with only shorter repeat alleles (Kruskal-Wallis $\chi^2 = 0.02$; $df = 1$; $P = 0.900$).

To clarify further the relationship between repeat length and breast cancer risk, an analysis of trend was undertaken by defining three groups. These groups were defined by having a shorter *AIB1* allele of <27 (*i.e.,* "short" alleles), 27 or 28 (*i.e.,* "medium" alleles), and >28 (*i.e.,* "long" alleles). In this analysis, longer *AIB1* repeat alleles were again associated with higher breast cancer risk. After adjusting for age at first live birth, parity, smoking status, and year of birth, we estimated this increased risk to be OR, 1.96 (95% CI, 1.25–3.08; *P* for trend = 0.0036). This corresponded to a 2-fold increase in breast cancer risk for each unit increase from the short to the medium to the long repeat-length groups.

Because *AIB1* is thought to enhance hormone-dependent transcription, we also evaluated the effect of the *AIB1* genotype across surrogates of endogenous hormone exposure as measured by reproductive history. As shown in Table 2, we observed no statistically significant effect of parity alone (nulliparous *versus* parous) or age at menarche (≥13 *versus* <13 years) on breast cancer risk in this sample. However, women who had a late age at first live birth (≥30 years) or were nulliparous were at significantly increased risk of breast cancer compared with women who had their first live birth before age 30 (OR, 3.06; 95% CI, 1.52–6.16). We also found significant differences in breast cancer risk across strata defined by *AIB1* genotype and reproductive history (Table 3). An approximately additive interaction of age at first live birth and *AIB1* genotype was observed, with effects as high as an OR of 4.62 or 6.97 in women who carried only long *AIB1* repeat alleles (28 or 29 or more repeats, respectively) and who were nulliparous or had a late age at first live birth. These results were associated with significant tests for trend across levels of genotype and reproductive factors ($\chi^2_{TREND} = 5.93$; $P = 0.015$ and $\chi^2_{TREND} = 25.14$; $P < 0.001$ for repeat cutpoints of 28 and 29 alleles, respectively). There was no significant trend in the relationship between genotype and age at menarche on probability of having breast cancer. However, an increase in probability of having breast cancer was observed in women with at least one long (≥29) repeat-length allele in both early (OR, 3.06; 95% CI, 1.31–7.17) and late (OR, 2.34; 95% CI, 1.10–4.96) age at menarche. Parous women who had at least one long *AIB1* repeat allele also had a significantly increased probability of having breast cancer compared with parous women without long *AIB1* repeat alleles (OR, 2.96; 95% CI, 1.65–5.31), but no similar effect was observed in nulliparous women.

Our sample consisted of 370 (82.6%) *BRCA1* mutation carriers and 78 (17.4%) *BRCA2* mutation carriers. Considering only the *BRCA1* mutation carriers in a subset analysis, the significant relationships reported in Table 3 persisted. For example, compared with the reference group of women with an age at first live birth before 30 and no *AIB1* allele of 29 or more repeats, the OR for women with at least one allele of 29 or more repeats and an early age at first live birth was 3.06 (95% CI, 1.54–6.08), the OR for women with no allele of 29 or more repeats who were nulliparous or had a late age at first live birth was 3.50 (95% CI, 1.57–7.80), and the OR for women with at least one allele of 29 or more repeats who were nulliparous or had a late age at first live birth was 9.25 (95% CI, 1.68–50.78). These results strongly parallel those presented in Table 3, as do the other associations of *AIB1* and age at menarche or parity in the subset of *BRCA1* mutation carrier (results not shown). The relatively small number of *BRCA2* mutation carriers precluded analysis of this subset alone. Therefore, the results for *BRCA1* mutation carriers, who represented the majority of the study sample, closely reflected the results in the sample as a whole.

DISCUSSION

Our results imply that endocrine factors acting through reproductive hormone exposure and the *AIB1* signaling pathway may be associated with increased breast cancer risk in women who have inherited germ-line mutations in *BRCA1/2*. Hormonal factors may modulate *BRCA1*-associated breast cancer risk by acting directly on the normal mammary epithelium to alter the initiation or progression of breast cancer. Alternatively these factors may act through endocrine mechanisms to alter the levels of circulating hormones or via paracrine mechanisms involving effects mediated by hormonally responsive cells in the mammary epithelium or stroma.

It has been reported that *AIB1* acts in concert with the ERs or ARs to mediate its endocrine effects. Anzick *et al.* (10) reported that *AIB1* mediates the endocrine-signaling effects of estrogen exposure on breast epithelial or tumor cells. However, the majority of *BRCA1*-associated tumors are ER-negative (20). Therefore, our results suggest that if the effect of *AIB1* in *BRCA1* mutation carriers is mediated through ER, these effects must occur either when the premalignant breast cells express ER or alternatively through effects on AR or some other related hormone-signaling pathways. Bevan *et al.* (13) reported that the glutamine-rich region of the AIB1-related coactivator SRC1

Table 3 *Results of analyses of interaction of* AIB1 *and other factors*

Factor	Genotype[c]	OR[a] (95% CI) associated with *AIB1* polyglutamine length cutpoint[b] at:	
		28 repeats	29 repeats
Age at first live birth <30	0	1.0[d]	1.0[d]
	1	1.82 (1.11–2.98)	3.04 (1.66–5.55)
Age at first live birth ≥30 or nulliparous	0	4.95 (1.66–14.75)	3.46 (1.67–7.14)
	1	4.62 (2.02–10.56)	6.97 (1.71–28.43)
Menarche <13	0	1.0[d]	1.0[d]
	1	2.07 (1.06–4.05)	3.06 (1.31–7.17)
Menarche ≥13	0	1.14 (0.54–2.40)	0.86 (0.56–1.35)
	1	1.48 (0.78–2.80)	2.34 (1.10–4.96)
Parous	0	1.0[d]	1.0[d]
	1	1.82 (1.14–2.90)	2.96 (1.65–5.31)
Nulliparous	0	1.67 (0.39–7.27)	0.65 (0.25–1.70)
	1	0.83 (0.30–2.34)	1.32 (0.24–7.09)

[a] Adjusted for year of birth, age at first live birth, age at menarche, parity, and smoking, except where that variable was involved in the interaction analysis.

[b] *AIB1* cutpoint made at polyglutamine repeat length longer than or equal to the indicated number, compared with genotypes containing only shorter number of repeats.

[c] Genotype 0, individuals with alleles shorter than the cutpoint of interest; genotype 1, individuals with alleles as long or longer than the cutpoint of interest.

[d] Reference group.

interacts directly with AR and is necessary and sufficient for enhancement of androgen-signaling capacity. Irvine *et al.* (7) reported that AR transactivation activity is dependent on AIB1 as a coactivator. We have reported previously that the CAG repeat polymorphism in exon 1 of *AR* modulates *BRCA1*-associated breast carcinogenesis (21), and suggests that androgen signaling may be involved in *BRCA1*-associated breast cancer risk. Both that study and the present report suggest that genes involved in endocrine signaling may modulate *BRCA1/2*-associated breast carcinogenesis. We are currently evaluating whether a combination of *AIB1* and *AR* genotypes interact to modify *BRCA1/2*-associated breast cancer risk. However, more definitive information about the mechanisms underlying the association reported here must await the results of studies that define the relationship of the *AIB1* polyglutamine polymorphism and *AIB1* function.

We also report an effect of reproductive history on breast cancer risk in this sample. Breast cancer risk in the general population is affected by reproductive history (22). It is therefore plausible that these factors may also modify the occurrence of breast cancer in *BRCA1/2* mutation carriers. Narod *et al.* (2) studied the effect of reproductive history in 333 women inferred from genetic linkage studies to be *BRCA1* mutation carriers. They reported that breast cancer risk was modified only by low parity. Jernström *et al.* (4) reported a 70% increase in the risk of breast cancer among women diagnosed before age 40 who carried *BRCA1/2* mutations. This result is in contrast to the statistically nonsignificant association we observed with parity alone in the present sample of breast cancer cases 22–74 years of age at the time of diagnosis. Using a small sample of putative *BRCA1* mutation carriers, Chang-Claude *et al.* (23) did not identify any significant reproductive factors as breast cancer risk modifiers, although early age at menarche and late age at first live birth provided suggestive evidence for a modifying effect. In the present study, we infer that women who are either nulliparous or have had a late age at first live birth are at increased breast cancer risk, particularly if they carry an *AIB1* genotype with 29 or more CAG repeats. These results suggest that endogenous exposure to hormones, as measured by reproductive history, affects *BRCA1/2*-associated breast cancer risk, and that the effects of age at first live birth and parity may be relevant when considering breast cancer risk in these women.

There were a number of limitations in the present analyses. First, the participants carried a variety of *BRCA1/2* mutations, and we could not evaluate the effect of *BRCA1/2* mutation type or location on the present results. However it is unlikely that the heterogeneous collection of *BRCA1/2* mutations substantially influenced our inferences, and given the extreme heterogeneity of mutations in *BRCA1/2*, it is unlikely that a comprehensive analysis of the effect of mutation location or type could be carried out. Furthermore, by not limiting the analyses to a particular class of mutations, the present results may be applicable to the general population of *BRCA1/2* mutation carriers from high-risk families. An additional limitation is that some individuals in the families studied here may have been excluded because they had died or were otherwise unable to participate in this research. As a result, the present results do not allow us to distinguish whether this effect implicates *AIB1* as an independent breast cancer risk factor or as a modifier of *BRCA1/2*-associated breast carcinogenesis.

Data are now becoming available about the function of specific *AIB1* alleles that complement knowledge of the importance of this region in steroid hormone signaling (13). Patel *et al.* (24) found that the polyglutamine repeat length was associated with bone mineral density and that this association remained significant even when ER genotypes were considered. More recently, Jernström *et al.* (25) reported that *AIB1* polyglutamine repeat length was associated with IGF-I levels among women who used oral contraceptives. Circulating IGF-I is strongly influenced by exogenous estrogen. This finding is consistent with that of Wang *et al.* (26), who demonstrated that *AIB1*-deficient mice had altered IGF-I expression. The finding that *AIB1* polyglutamine repeat length may mediate the effect of exogenous estrogen to modulate IGF-I levels suggests that this polymorphism is functionally associated with estrogen signaling. Jernström *et al.* (25) concluded that the *AIB1* polyglutamine repeat lengths reported here as being associated with breast cancer risk are more potent coactivators of estrogen signaling than others. These results support our inference that the length of the polyglutamine repeat is associated with *BRCA1/2*-associated breast cancer risk.

Analogous to studies of the CAG repeat polymorphism in *AR* (5), we created comparison groups based on the length of the polyglutamine repeats with the goal of studying genotype classes with longer or shorter repeat lengths. On the basis of the distribution of alleles shown in Table 1, it appeared that the primary source of the case-control differences is explained by the 29-repeat allele. In particular, the increased frequency of 29/29 genotype individuals was apparent in cases compared with controls, and there was a significant trend toward increasing risk with increasing repeat allele size (*i.e.,* <27, 27 or 28, and >28). Because other alleles (*i.e.,* those with <26, 27, or >29 polyglutamines) are rare, the creation of other genotype strata results in very small groups. Therefore, no statistically meaningful inferences could be made from an analysis of cutpoints other than those presented in Tables 2 and 3. As a result, it is also impossible to determine whether longer allele length is responsible for the reported effects, whether the effect is specific to the 29-repeat allele itself, or whether the 29-repeat allele is in linkage dysequilibrium with some other relevant (but unmeasured) allele. Although a number of comparisons using other categorizations could have been considered, these analyses would be largely underpowered to detect relevant effects and would result in an increased number of hypothesis tests. Therefore, we have limited our analyses to the most prevalent and statistically meaningful genotype comparisons.

Finally, our primary analyses evaluated women with germ-line mutations in *BRCA1* and *BRCA2* together. However, it is apparent that these two genes confer different clinical and molecular phenotypes, and that comparisons should be made for each gene independently. Although our study design accounted for the potential of confounding by *BRCA1 versus BRCA2* mutations by matching cases and controls by locus, analyses stratified on genotype were not possible as the majority of our sample (370 women, or 83%) consisted of *BRCA1* mutation carriers. However, we undertook a subset analysis of *BRCA1* mutation carriers only. In that subset, the OR effect of *AIB1* genotypes with 29 or more repeats was 3.06 (95% CI, 1.54–6.08) in women who were nulliparous or who had an early age at first live birth and 9.25 (95% CI, 1.68–50.78) in women who had a late age at first live birth. These results suggest that the effect is consistent among the subset of *BRCA1* mutation carriers in this sample. Additional evaluation should be undertaken to compare subsets of *BRCA1* and *BRCA2* mutation carriers separately and to explore why the effect of *AIB1* on breast cancer risk may differ between *BRCA1/2* mutation carriers and individuals in the general population (15).

We conclude that the length of the *AIB1* repeat may affect breast cancer risk in women who have inherited a germ-line *BRCA1/2* mutation, possibly through modulation of hormonal responses of the mammary epithelium. Our observations imply that germ-line mutations in *BRCA1/2* may be necessary to explain the Mendelian pattern of cancer in some families, but may not be sufficient to completely describe the interindividual variability in the age-specific risk of cancer. The ability to effectively apply risk-prediction or cancer-prevention strategies in *BRCA1/2* carriers may therefore depend on knowledge of risk-modifying factors in addition to *BRCA1/2* mutation

status. However, additional research about *AIB1* genotype will be required before it is possible to make clinical decisions about breast cancer risk, surveillance, or prevention among *BRCA1/2* mutation carriers.

REFERENCES

1. Easton, D. F., Ford, D., and Bishop, D. T. Breast and ovarian cancer incidence in *BRCA1* mutation carriers. Breast Cancer Linkage Consortium. Am. J. Hum. Genet., *56:* 265–271, 1995.

2. Narod, S. A., Goldgar, D., Cannon-Albright, L., Weber, B. L., Moslehi, R., Ives, E., Lenoir, G., and Lynch, H. Risk modifiers in carriers of *BRCA1* mutations. Int. J. Cancer, *64:* 394–398, 1995.

3. Ford, D., Easton, D. F., Stratton, M., Narod, S., Goldgar, D., Devilee, P., Bishop, D. T., Weber, B., Lenoir, G., Chang-Claude, J., Sobol, H., Teare, M. D., Struewing, J., Arason, A., Scherneck, S., Peto, J., Rebbeck, T. R., Tonin, P., Neuhausen, S., Barkardottir, R., Eyfjord, J., Lynch, H., Ponder, B. A., Gayther, S. A., Zelada-Hedman, M., and the Breast Cancer Linkage Consortium. Genetic heterogeneity and penetrance analysis of the *BRCA1* and *BRCA2* genes in breast cancer families. Am. J. Hum. Genet., *62:* 676–689, 1998.

4. Jernström, H., Lerman, C., Ghadirian, P., Lynch, H. T., Weber, B., Garber, J., Daly, M., Olopade, O. I., Foulkes, W. D., Warner, E., Brunet, J. S., and Narod, S. A. Pregnancy and risk of early breast cancer in carriers of BRCA1 and BRCA2. Lancet, *354:* 1846–1850, 1999.

5. Rebbeck, T. R., Levin, A. M., Eisen, A., Snyder, C., Watson, P., Cannon-Albright, L., Isaacs, C., Olopade, O., Garber, J. E., Godwin, A. K., Daly, M. B., Narod, S. A., Neuhausen, S. L., Lynch, H. T., and Weber, B. L. Breast cancer risk after bilateral prophylactic oophorectomy in BRCA1 mutation carriers. J. Natl. Cancer Inst., *91:* 1475–1479, 1999.

6. Park, J. J., Irvine, R. A., Buchanan, G., Koh, S. S., Park, J. M., Tilley, W. D., Stallcup, M. R., Press, M. F., and Coetzee, G. A. Breast cancer susceptibility gene 1 (*BRCA1*) is a coactivator of the androgen receptor. Cancer Res., *60:* 232–236, 2000.

7. Irvine, R. A., Ma, H., Yu, M. C., Ross, R. K., Stallcup, M. R., and Coetzee, G. A. Inhibition of p160-mediated coactivation with increasing androgen receptor polyglutamine length. Hum. Mol. Genet., *9:* 267–274, 2000.

8. Yoshida-Komiy, H., Deng, C., and O'Malley, B. W. The steroid receptor coactivator SRC(p/CIP/RAC3/*AIB1*/ACTR/TRAM-1) is required for normal growth, puberty, female reproductive function, and mammary gland development. Proc. Natl. Acad. Sci. USA, *97:* 6379–6384, 2000.

9. Guan, X. Y., Xu, J., Anzick, S. L., Zhang, H., Trent, J. M., and Meltzer, P. S. Hybrid selection of transcribed sequences from microdissected DNA: isolation of genes within amplified region at 20q11–q13.2 in breast cancer. Cancer Res., *56:* 3446–3450, 1996.

10. Anzick, S. L., Konen, J., Walker, R. L., Azorosa, D. O., Tanner, M. M., Guan, X. Y., Sauter, G., Kallioniemi, O. P., Trent, J. M., and Meltzer, P. S. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science (Wash. DC), *277:* 965–968, 1997.

11. Bautista, S., Valles, H., Walker, R. L., Anzick, S., Zeillinger, R., Melktzre, P., and Theiller, C. In breast cancer, amplification of the steroid receptor coactivator gene *AIB1* is correlated with estrogen and progesterone receptor positivity. Clin. Cancer Res., *4:* 2925–2929, 1998.

12. Hayashi, Y., Yamamoto, M., Ohmori, S., Kikumori, T., Imai, T., Funahashi, H., and Seo, H. Polymorphism of homopolymeric glutamines in coactivators for nuclear hormone receptors. Endocr. J., *46:* 279–284, 1999.

13. Bevan, C. L., Hoare, S., Claessens, F., Heery, D. M., and Parker, M. G. The AF1 and AF2 domains of the androgen receptor interact with distinct regions of SRC1. Mol. Cell. Biol., *19:* 8383–8392, 1999.

14. Spurdle, A. B., Dite, G. S., Chen, X., Mayne, C. J., Southey, M. C., Batten, L. E., Chy, H., Trute, L., McCredie, M. R., Giles, G. G., Armes, J., Venter, D. J., Hopper, J. L., and Chenevix-Trench, G. Androgen receptor exon 1 CAG repeat length and breast cancer risk in women before age forty years. J. Natl. Cancer Inst., *91:* 961–966, 1999.

15. Haiman, C. A., Hankinson, S. E., Spiegelman, D., Colditz, G. A., Willett, W. C., Speizer, F. E., Brown, M., and Hunter, D. J. Polymorphic repeat in AIB1 does not alter breast cancer risk. Breast Cancer Res., *2:* 378–385, 2000.

16. Shirazi, S. K., Bober, M. A., and Coetzee, G. A. Polymorphic exonic CAG microsatellites in the gene amplified in breast Cancer (*AIB1* gene). Clin. Genet., *54:* 102–103, 1998.

17. La Spada, A. R., Wilson, E. M., Lubahn, D. B., Harding, A. E., and Fischbeck, K. H. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature (Lond.), *352:* 77–79, 1991.

18. Kazemi-Esfarjani, P., Trifiro, M. A., and Pinsky, L. Evidence for a repressive function of the long polyglutamine tract in the human androgen receptor: possible pathogenetic relevance for the (CAG)n-expanded neuronopathies. Hum. Mol. Genet., *4:* 523–527, 1995.

19. Landis, J. R., Heyman, E. R., and Koch, G. G. Average partial association in three-way contingency tables: a review and discussion of alternative tests. Int. Stat. Rev., *46:* 237–254, 1978.

20. Karp, S. E., Tonin, P. N., Begin, L. R., Martinez, J. J., Zhang, J. C., Pollak, M. N., and Foulkes, W. D. Influence of BRCA1 mutations on nuclear grade and estrogen receptor status of breast carcinoma in Ashkenazi Jewish women. Cancer (Phila.), *80:* 435–441, 1997.

21. Rebbeck, T. R., Kantoff, P. A., Krithivas, K., Godwin, A. K., Daly, M. B., Narod, S. A., Garber, J. E., Weber, B. L., and Brown, M. Modification of *BRCA1*-associated breast cancer penetrance by androgen receptor CAG repeat length variants. Am. J. Hum. Genet., *64:* 1371–1377, 1999.

22. Ewertz, M., Duffy, S. W., Adami, H. O., Kvale, G., Lund, E., Meirik, O., Mellemgaard, A., Soini, I., and Tulinius, H. Age at first birth, parity and risk of breast cancer: a meta-analysis of 8 studies from the Nordic countries. Int. J. Cancer, *46:* 597–603, 1990.

23. Chang-Claude, J., Becher, H., Eby, N., Bastert, G., Wahrendorf, J., and Hamann, U. Modifying effect of reproductive risk factors on the age at onset of breast cancer for German BRCA1 mutation carriers. J. Cancer Res. Clin. Oncol., *123:* 272–279, 1997.

24. Patel, M. S., Cole, D. E., Smith, J. D., Hawker, G. A., Wong, B., Trang, H., Vieth, R., Meltzer, P., and Rubin, L. A. Alleles of the estrogen receptor-α gene and an estrogen receptor cotranscriptional activator gene, amplified in breast cancer-1 (*AIB1*), are associated with quantitative calcaneal ultrasound. J. Bone Miner. Res., *15:* 2231–2239, 2000.

25. Jernström, H., Chu, W., Vesprini, D., Tao, Y., Majeed, N., Deal, C., Pollak, M., and Narod, S. A. Genetic factors related to racial variation in plasma levels of insulin-like growth factor-1: implications for premenopausal breast cancer risk. Mol. Genet. Metab., *72:* 144–154, 2001.

26. Wang, Z., Rose, D. W., Hermanson, O., Liu, F., Herman, T., Wu, W., Szeto, D., Gleiberman, A., Krones, A., Pratt, K., Rosenfeld, R., Glass, C. K., and Rosenfeld, M. G. Regulation of somatic growth by the p160 coactivator p/CIP. Proc. Natl. Acad. Sci. USA, *97:* 13549–13454, 2000.