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POLYGLUTAMINE REPEAT LENGTH IN THE *AIB1* GENE MODIFIES BREAST CANCER SUSCEPTIBILITY IN *BRCA1* CARRIERS

Luna KADOURI[1]*, Zsofia KOTE-JARAI[2], Douglas F. EASTON[3], Ayala HUBERT[1], Rifat HAMOUDI[2], Benjamin GLASER[4], Dvorah ABELIOVICH[4], Tamar PERETZ[1] and Rosalind A. EELES[2,6]

[1]*Sharett Institute of Oncology, Hebrew University-Hadassah Medical Center, Jerusalem, Israel*
[2]*Section of Cancer Genetics, The Institute of Cancer Research, Sutton, United Kingdom*
[3]*Cancer Research UK Genetic Epidemiology Unit, Strangeways Research Laboratory, Cambridge, United Kingdom*
[4]*Human Genetic Laboratories, Hebrew University-Hadassah Medical Center, Jerusalem, Israel*
[5]*The Royal Marsden NHS Trust, Sutton, United Kingdom*

Variation in the penetrance estimates for *BRCA1* and *BRCA2* mutation carriers suggests that other factors may modify cancer risk from specific mutations. One possible mechanism is an epigenetic effect of polymorphisms in other genes. Genes involved in hormonal signal transduction are possible candidates. The *AIB1* gene, an estrogen receptor (ER) coactivator, is frequently amplified in breast and ovarian tumors. Variation of a CAG repeat length has been reported within this gene that encodes a polyglutamine repeat in the C-terminus of the protein. Three hundred eleven *BRCA1/2* mutation carriers (257 were of Ashkenazi origin) were genotyped for the *AIB1* polyglutamine repeat. Relative risks (RR) were estimated using a maximum likelihood approach. The estimated breast cancer (BC) RR per average repeat length adjusted for population type (Ashkenazi *vs.* non-Ashkenazi) was 1.15 (95% CI = 1.02–1.30; $p = 0.01$) for *BRCA1/2* carriers, and 1.25 (95% CI = 1.09–1.42; $p = 0.001$) when analysis was restricted to *BRCA1* carriers. RR of BC was 1.17 (95% CI = 0.91–1.74), for individuals with 2 alleles ≥29 polyglutamine repeats and 0.78 (95% CI = 0.50–1.16) for those with at least 1 allele of ≤26 repeats, compared to individuals with the common genotypes 28;28, 28;29 or 28;30. The corresponding BC RR in *BRCA1* mutation carriers was 0.55 (95% CI = 0.34–0.90) and 1.29 (95% CI = 0.85–1.96) in those with ≤26 and ≥29 repeats respectively ($p = 0.025$). These results indicate significant association of the risk for BC in carriers of *BRCA1* mutations with the polyglutamine chain of the AIB1 gene. Longer repeat length correlates with elevated risk, whereas in carriers of a shorter AIB1 allele BC risk was reduced. The *AIB1* polyglutamine length did not affect BC risk among *BRCA2* mutation carriers.

Key words: *BRCA1/2; breast cancer risk;* AIB1 *gene; polymorphism*

Carriers of a mutated *BRCA1* or *BRCA2* gene are at an increased risk of breast cancer (BC), however penetrance estimates are different in various study populations.[1–4] Breast cancer risk for BRCA1/2 carriers varied from 70% by age 70 in studies based on families with numerous cases[1,2] to 37–56% in population based studies.[3,4] Modification of the risk by other genes or environmental factors clustering in families probably explains most of this difference. When considering the former possibilities genes involved in hormonal signal transduction are of particular interest in mammary carcinogenesis both in *BRCA1/2* mutation carriers and in the general population.

The *AIB1* gene (amplified in breast cancer-1) was cloned using chromosome microdissection and hybrid selection of the 20q region that is often amplified in BC.[5] As a member of the steroid receptor coactivator (SRC-1) family, it interacts with estrogen-receptor-α (ER-α) in a ligand-dependent manner.[5] It has been demonstrated that AIB1 is a phosphoprotein that can be phosphorylated by MAPKs, enhancing its activity. The stimulated protein then recruits the p300 and associated histone acetyltransferase activity.[6]

AIB1 is amplified and highly expressed in BC and ovarian cancer (OC), especially in ER positive tumors.[7] *AIB1* mRNA, however, is also overexpressed in high grade tumors that are ER and PR negative, and positive for p53 and HER2/neu staining.[8] Gene amplification is also found in pancreatic cancer cell lines[9] and gastric cancers[10] that suggests a role for AIB1 in other, non ER-related, signal transduction pathways. It has also been found to have an effect on IGF-1 response, suggesting that it might also have a role in somatic growth.[11]

The glutamine codons, CAG and CAA encode a glutamine rich domain in the C-terminus of the AIB1 protein.[12,13] It contains 2 polymorphic stretches of CAG repeats separated by a CAA repeat. The functional significance of this repeat is unknown. If the repeat length altered the protein function it could modulate the transcriptional activity of ER, however, and could modify BC susceptibility. Elevated BC risk associated with a longer repeat was reported in *BRCA1/2* carriers[14] but not in the general population.[15]

We assessed the effect of the polyglutamine repeat polymorphism in the *AIB1* gene on BC risk in *BRCA1/2* mutation carriers, mainly of Ashkenazi origin.

MATERIAL AND METHODS

Study population

Blood samples from 325 *BRCA1/2* carriers were identified through 2 centers: 243 were collected through the oncology department and the cancer genetic clinic in the Hadassah Medical Centre in Jerusalem, Israel and 82 through the cancer genetic clinic in the Royal Marsden NHS Trust, London, UK. Individuals were tested on the basis of a family history of breast or ovarian cancer or on the basis of their Ashkenazi origin. All but 1 of the cases from Jerusalem (that carried a BRCA2 mutation; 8558delA), were carriers of 1 of the 3 Ashkenazi founder mutations (*BRCA1* 185delAG: 134 cases, *BRCA1* 5382insC: 76 cases, *BRCA2* 6174delT: 32 cases). The UK carriers included 23 carriers of Ashkenazi founder mutations (1 individual carried both a 185delAG and 6174delT) and 59 other mutations (48 *BRCA1*; 11 *BRCA2;* for list of mutations see Appendix).

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*Correspondence to: Sharett Institute of Oncology, Hadassah Medical Center, Jerusalem, Israel, 91120. Fax: +972-0-2-6427485.
E-mail: luna@hadassah.org.il

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Genotyping

Genomic DNA was extracted according to standard protocols, and used as a template for the PCR reaction. The region of the *AIB1* gene that includes the polymorphic CAG repeat was amplified using primers, forward: 5′-TCCGACAACAGAGGGTGGC-TATG-3′ and reverse: 5′-TTAGGAGGTGGGCTGAAGGCCTG-3′of which the forward primer was labeled fluorescently. Typically 25 ng of genomic DNA was used in each PCR reaction of 15 μl. PCR cycling consisted of 35 cycles of 94°C, 64°C and 68°C for 30 sec for each stage after an initial 10 min at 95°C. Each reaction contained 2.0 mM $MgCl_2$ and 1 U of Amplitaq Gold (Perkin-Elmer, Norwalk, CT). After PCR amplification the repeat length was analyzed using an ABI 377 automated DNA Sequencer. The size of the amplified products was determined relative to size standards using Genescan and Genotyper Analysis software (Perkin-Elmer). Genotyping was carried out by 2 independent personnel who were blinded to the case-control status of the samples. Polymorphic polyglutamine repeat length containing 20–31 repeats of the trinucleotide glutamine codons (CAA, CAG) was scored as described previously.[14,15] Several samples were sequenced to confirm the repeat length as established by the ABI 377 Genotyper. Blinded quality control samples were inserted to validate genotype scoring accuracy.

Statistical analysis

Six of the 325 carriers were excluded from all analyses; 2 for whom the date of birth was unknown, and 4 individuals recorded as affected but for whom the age at diagnosis was unknown. A further 8 individuals could not be genotyped so that the final analysis included data from 311 mutation carriers.

The analyses presented here examined the association between breast cancer risk and genotype. For simplicity the analyses were based on the first occurring cancer (*i.e.*, all individuals were censored at the first cancer). With this classification there were 195 breast cancer cases (18 also had also ovarian cancer) and 116 individuals unaffected with breast cancer at censoring (39 had ovarian cancer). Individuals unaffected with breast cancer were censored either at date of ovarian cancer diagnosis, date of prophylactic surgery or date of last follow up.

The effects of *AIB1* genotypes on breast cancer risk in mutation carriers were evaluated using a maximum likelihood approach, based on the likelihood of the observed phenotypic and genotypic data conditional on the phenotypic data. This approach provides consistent estimates of the risk parameters despite the non-random ascertainment of subjects with respect to phenotype. In this analysis, the incidence of breast in individuals with genotype Category j was assumed to be given by:

$$\lambda j(t) = \lambda_0(t) \exp(\beta_j)$$

where $\exp(\beta_j)$ is the rate ratio for breast cancer in Category j and $\lambda_0(t)$ are the age-specific incidence rates in the baseline category. We estimated the rate ratios for different genotype categories. $\lambda_0(t)$ was chosen so that the overall breast cancer incidence rates in carriers, averaged over all genotypic categories, agreed with previously derived incidence rates for *BRCA1* and *BRCA2* carriers. These estimates were taken from the recent meta-analysis of cancer risks in *BRCA1* and *BRCA2* carriers from population-based studies.[16] In our study, breast cancer estimated risks are moderately lower than the estimates derived by the BCLC studies of high risk families[1,2] and also slightly lower than the estimates of a population based study conducted in the Ashkenazi population.[3] These risk estimates is applicable to our carrier sample that is mainly composed of a population based series. Two types of analyses were carried out. In the first analysis we treated allele size as a continuous covariate, and estimated a single log-risk ratio parameter for the increase in risk per unit length. In the main analysis, we used the average of the allele lengths on the 2 chromosomes as the covariate (in effect assuming an additive effect of the 2 alleles) but we also carried out analyses based on the longer and shorter allele lengths. In the second type of analysis, we classified AIB1 alleles according to the shorter allele into 3 categories: ≤26 repeats, 28 repeats and ≥29 repeats, and estimated risk ratios based on the category of the shorter repeat length. In each analysis, we estimated the log-risk ratio parameters together with population allele frequencies. The latter were estimated separately for the Ashkenazi and non-Ashkenazi groups, to allow for possible differences in *AIB1* allele frequencies. These analyses were carried out using the program MENDEL.[17] We also carried out analysis using the previously described score test[18] that gave similar results (not shown).

RESULTS

Table I shows the distribution of *AIB1* polyglutamine repeat length in the Ashkenazi carriers and British non-Ashkenazi population. When genotypes are categorized by the shorter allele, the most common alleles were 26, 28 and 29 repeats with frequency of 24.3%, 60.1% and 15.6% in the Ashkenazi population and 16.3%, 54.5% and 29.1% in the British population. These frequencies are comparable to those reported in other populations.[12–15] Allele lengths were however, significantly shorter, on average, in the Ashkenazi populations than the U.K. carriers ($p_{trend} = 0.0007$). Extremely short repeats of 22 repeats were limited to the Ashkenazi Population (7/257; 2.7%) and a carrier of a 31 polyglutamine repeat allele was identified among non-Ashkenazi BRCA carriers.

The frequencies of AIB1 genotypes according to disease status in BRCA1 and BRCA2 carriers is shown in Table II. Cut-off points that were used in the categorized risk analyses (Table III) are marked in the table.

The estimated BC relative risk (RR) in *BRCA1/2* carriers by average polyglutamine repeat length as a continuous variable (Table II) was 1.15 (95%CI = 1.02–1.30, $p = 0.01$) per repeat unit. Similar effects were observed when analysis was based on the length of the longer or the shorter allele. The estimated effect was stronger for *BRCA1* carriers (RR = 1.25; 95% CI = 1.09–1.42), with no significant effect in *BRCA2* carriers (RR = 0.93; 95% CI = 0.78–1.12; $p = 0.013$ for difference in RRs).

To further assess the contribution of polyglutamine repeat length to breast cancer risk, we categorized individuals by the smaller repeat length. In comparison with individuals with the "common" 28/28, 28/29, 28/30 genotypes, RR for carriers of 2 alleles ≥29 repeats was 1.17 (95% CI = 0.91–1.74), whereas carriers with 1 allele ≤26 repeats had a reduced risk (RR = 0.78; 95% CI = 0.52–1.16). In *BRCA1* carriers, BC RRs were 1.29 (95% CI = 0.85–1.96) and 0.55 (95% CI = 0.34–0.90) in individuals with 2 alleles of ≥29 and 1 allele of ≤26 repeats respectively ($p = 0.025$).

The mean age at onset of the BC cases was not significantly related to repeat length; the mean age of onset was 41.2 years for

TABLE I – GENOTYPE DISTRIBUTION OF THE POLYGLUTAMINE REPEAT POLYMORPHISM IN THE *AIB1* GENE IN ASHKENAZI AND NON-ASHKENAZI *BRCA1/2* MUTATION CARRIERS

Polyglutamine repeat length	Ashkenazi[1] *n* (%)	Non-Ashkenazi[2] *n* (%)
22/28	4 (1.6)	0
22/29	3 (1.2)	0
26/26	3 (1.2)	0
26/28	31 (12.1)	5 (9.1)
26/29	21 (8.2)	3 (5.4)
26/31	0	1 (1.8)
28/28	47 (18.3)	6 (10.9)
28/29	105 (41.0)	23 (41.8)
28/30	2 (0.8)	1 (1.8)
29/29	40 (15.6)	15 (27.3)
29/30	0	1 (1.8)

[1]$n = 256$.–[2]$n = 55$.

TABLE II – FREQUENCIES OF POLYGLUTAMINE REPEAT LENGTHS IN THE *AIB1* GENE BY DISEASE STATUS IN *BRCA1* AND *BRCA2* CARRIERS[1]

Polyglutamine repeat length	*BRCA1* carriers (n = 222)		*BRCA2* carriers (n = 88)	
	BC + (n = 138)	BC − (n = 84)	BC + (n = 57)	BC − (n = 31)
22/28	2 (1.4)	1 (1.2)	1 (1.8)	0
22/29	0	1 (1.2)	2 (3.5)	0
26/26	2 (1.4)	1 (1.2)	0	0
26/28	10 (7.2)	12 (14.3)	8 (14)	6 (19.4)
26/29	8 (5.8)	8 (9.5)	6 (10.5)	2 (6.4)
26/31[2]	1 (0.7)	0	0	0
28/28	23 (16.7)	16 (20.2)	8 (14)	7 (22.6)
28/29	57 (41.3)	30 (34.5)	25 (43.9)	14 (45.2)
28/30[2]	1 (0.7)	2 (2.4)	0	0
29/29	34 (24.6)	12 (14.3)	7 (12.3)	2 (6.4)
29/30[2]	0	1 (1.2)	0	0

[1]The individual carrying a BRCA1&2 mutation is excluded. Values are n (%). BC+, affected with breast cancer; BC−, unaffected with breast cancer.–[2]Cutpoints used for the risk analysis by categorised AIB1 genotypes as presented in Table III: ≤26, = 28, ≥29.

TABLE III – RISK FOR BREAST CANCER IN *BRCA1/2* CARRIERS ASSOCIATED WITH POLYGLUTAMINE REPEAT LENGTH IN THE *AIB1* GENE

	BRCA1/2 carriers		*BRCA1* carriers		*BRCA2* carriers	
	RR (95% CI)	p	RR (95% CI)	p	RR (95% CI)	p
AIB1 polyglutamine repeat length as a continuous covariate						
Average repeat length	1.15 (1.02–1.30)	0.01	1.25 (1.09–1.42)	0.001	0.93 (0.78–1.12)	
Shorter allele	1.17 (1.02–1.36)	0.03	1.35 (1.13–1.60)	0.0005	0.86 (0.70–1.07)	0.17
Longer allele	1.36 (1.00–1.85)	0.05	1.28 (0.93–1.74)	0.17	1.41 (0.83–2.40)	0.22
Length by specific allele grouping						
1 alleles ≤ 26	0.78 (0.52–1.16)[1]		0.55 (0.34–0.90)[1]		1.52 (0.88–2.63)[1]	
28;28, 28;29, 28;30	1.00 (0.80–1.25)		1.00 (0.77–1.30)		1.00 (0.69–1.45)	
2 alleles ≥ 29	1.17 (0.91–1.74)	0.10[2]	1.29 (0.85–1.96)	0.025[2]	0.77 (0.34–1.75)	0.13[2]

[1]FAR confidence limits (see Methods).–[2]2df likelihood ratio test

TABLE IV – FREQUENCIES OF CATEGORIZED POLYGLUTAMINE REPEAT LENGTHS BY DISEASE STATUS AND AGE AT BREAST CANCER ONSET IN *BRCA1/2* CARRIERS

AIB1 repeats	*BRCA1/2* carriers (n = 311)	
	BC + Age at BC onset[2]	BC −
1 allele ≤ 26	40 (21)/42.0	31 (27)
28;28, 28;29, 28;30	114 (58)/41.3	70 (60)
2 alleles ≥ 29	41 (21)/41.2	15 (13)

[1]BC+, affected with breast cancer; BC−, unaffected with breast cancer. Values are n (%).–[2]Average age at breast cancer onset for individuals by *AIB1* CAG repeats grouping (years).

those with 2 alleles of ≥29 repeats, compared to 42.0 years for cases with at least 1 allele of ≤26 repeats (Table IV, $p_{\text{trend}} = 0.11$ in age at onset with average repeat length).

DISCUSSION

We have found a significant association between *AIB1* polyglutamine repeat length and breast cancer risk in *BRCA1* gene mutation carriers and a similar but non-significant association in *BRCA2* mutation carriers. The association was seen with the length of both the longer and the shorter allele, but was most significant when analyzed in terms of the average allele length. The results of the categorical analysis were also consistent with an effect of allele length, with a higher risk in carriers of 2 alleles of ≥29 repeats and a lower risk of approximately 2-fold in carriers of at least 1 allele of ≤26 repeats. These results would be consistent with a recessive type model, under which the length of the longer allele determines risk. A "dosage" type model in which each allele contributes to risk cannot be ruled out.

A study by Rebbeck *et al.*[14] has suggested a very similar association between the longer *AIB1* alleles and BC risk of 1.59 (95% CI = 1.5–2.9) and 2.85 (95% CI = 1.64–4.96) in women with at least 28 or 29 repeat lengths respectively in *BRCA1/2* carriers. An interaction with reproductive history of nulliparity and later age at first live birth was also found. They did not report separate analyses for *BRCA1* and *BRCA2* carriers.

Modifiers of cancer risk in *BRCA1/2* mutation carriers reported previously include the *H-ras* minisatellite locus, rare alleles of which were reported to be associated with ovarian cancer risk.[20] Longer length of the CAG repeats in the *AR* gene elevate breast cancer risk in *BRCA1* carriers,[18,21] whereas in *BRCA2* carriers BC risk was associated with a rare polymorphism in the *RAD51* gene.[22,23] In these studies, however, the proportion of carriers in the high-risk category is relatively low. In contrast, the *AIB1* high-risk category may be much larger. For example, the low and high risk categories in our analysis (1 ≤26 repeat allele and 2 ≥29 repeat alleles respectively) both have population frequencies of approximately 20%. The clinical implication of these findings are still limited, however it might provide additional guideline for risk estimation and consultation for *BRCA1* carriers.

The functional effect of the polyglutamine chain of the *AIB1* gene is currently unknown. Polyglutamine repeats are often identified in transcription factors and associated with transcriptional activity. The polyglutamine repeat length variation in several genes was associated with various neurological diseases.[24–26] The polyglutamine repeat in the *AR* gene has been associated with prostate cancer[27] and male breast cancer risk,[28] perhaps due to increased level of transactivation of the androgen receptor with shorter repeat alleles.[29] It has also been demonstrated that polyglutamine chains *per se* can activate transcription when fused to a yeast GAL4 DNA binding domain. Furthermore, there is evidence that the transactivation is affected by the length of the repeat.[30] A functional role for the *AIB1* polyglutamine repeat length polymorphism is suggested by 2 studies published recently. Patel *et al.*[31] reported association with bone mineral density, implying an effect on ER signaling pathways. IGF-I blood levels were also associated with the repeat length among oral contraceptives users.[32] Thus, it

is reasonable to speculate that the polyglutamine repeat length in the *AIB1* gene could have a significant effect on the protein function, perhaps altering the coactivation of the ER and hence modifying hormone-induced cancer risk.

Frequent amplification and overexpression of the *AIB1* gene was found, especially in ER and PR positive tumors that are rarely found in *BRCA1* carriers.[7] Contradictory findings of higher *AIB1* mRNA expression in association with ER and PR negativity, P53 and Her2/neu positivity were also reported.[8] Although the majority of *BRCA1* associated breast cancers are ER and PR negative,[33] the role of estrogen signaling in BC formation in this group is well established.[34,35] There is evidence that connects hormonal pathways to BRCA1/2 function. *BRCA1/2* gene expression is associated with steroid hormonal stimulation[36,37] and hormonal manipulation such as oophorectomy reduces BC risk in both *BRCA1* and *BRCA2* carriers.[35,36] Stronger evidence, however, links BRCA1 with hormonal signaling. BRCA1 was found to inhibit ER transcription activity in a breast cancer cell line,[35] and in the presence of mutated BRCA1, breast epithelial cell growth becomes independent of estrogen.[40] Therefore, the complex co-regulation of ER might be impaired in the presence of non-functional BRCA1 protein and may be more dependent on functional variations of other coactivators such as AIB1. This could explain the differential effect of *AIB1* repeat length in *BRCA1* mutation carriers *vs*. BRCA2 carriers.

In conclusion, our results suggest *AIB1* polyglutamine repeat lengths are associated with breast cancer risk among women with mutations in the *BRCA1* gene and to a lesser extent in *BRCA2* carriers. If these results are confirmed in further large series, genotyping of this locus could modify the risk predictions used in counseling of *BRCA1/2* mutation carriers. In addition, the differential effect we found in *BRCA1* carriers compared to *BRCA2* carriers may hint at the variation in molecular mechanisms underlying tumorigenesis in these 2 groups. Studies of the functional properties of the varying polyglutamine repeat lengths in the *AIB1* gene is, therefore, of great interest.

REFERENCES

1. Easton DF, Ford D, Bishop DT. Breast Cancer Linkage Consortium. Breast and ovarian cancer incidence in *BRCA1* mutation carriers. Am J Hum Genet 1995;56:265–71.
2. Ford D, Easton DF, Stratton MR, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Gharg-Claude J, Sobol H, Teare MD, et al. Genetic heterogeneity and penetrance analysis of BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet 1998;62: 676–89.
3. Struewing JP, Hartge P, Wacholdes S, Baker SM, Berlin M, McAdams M, Timmerman MM, Brody LC, Tucker MA. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 1997;336:1401–8.
4. Thorlacius S, Struewing JP, Hartge P, Olafsdottir GH, Sigvaldason H, Tryggvadottir L, Wacholder S, Tulinius H, Eyfjord JE. Population-based study of risk of breast cancer in carriers of *BRCA2* mutations. Lancet 1998;352:1337–9.
5. Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan X, Sauter G, Kallioniemi OP, Trent JM, Meltzer PS. AIB1, a steroid coactivator amplified in breast and ovarian cancer. Science 1997;277: 965–8.
6. Font de Mora J, Brown M. AIB1 is a conduit for kinase-mediated growth factor signaling to the estrogen receptor. Mol Cell Biol 2000; 20:5041–7.
7. Bautista S, Valles H, Walker RL, Anzick S, Zeillinger R, Melzer P, Theiller C. In breast cancer, amplification of the steroid receptor coactivator gene AIB1 is correlated with estrogen and progesterone receptor positivity. Clin Cancer Res 1998;4:2925–9.
8. Bouras T, Southey MC, Venter DJ. Overexpression of the steroid receptor coactivator AIB1 in breast cancer correlates with the absence of estrogen and progesterone receptors and positivity for p53 and HER2/new. Cancer Res 2001; 61: 903–7.
9. Ghadimi , Schrock E, Walker RL, Wangsa D, Jauho A, Meltzer PS, Ried T. Specific aberration and amplification of AIB1 in pancreatic cancer. Am J Pathol 1999;154:530–6.
10. Sakakura C, Hagiwara A, Yasuka R, Fujita Y, Nakanishi M, Masuda K, Kimura A, Nakamura Y, Inazawa J, Abe T, Yamagishi H. Amplification and over-statement of the AIB1 nuclear receptor co-activator gene in primary gastric cancer. Int J Cancer 2000;89:217–23.
11. Wang Z, Rose DW, Hermanson O, Liu F, Herman T, Wu W, Szeto D, Gleibeman A, Krones A, Pratt K, Rosenfeld R, Glass CK, Rosenfeld MG. Regulation of somatic growth by the p160 coactivator p/CIP. Proc Natl Acad Sci USA 2000;97:13549–54.
12. Shirazi SK, Bober MA, Coetzee GA. Polymorphic exonic CAG microsatellites in the gene amplified in breast cancer (*AIB1* gene). Clin Genet 1998;54:102–3.
13. Hayashi Y, Yamamoto M, Ohmori S, Kikumori T, Imai T, Funahashi H, Seo H. Polymorphism of homopolymorphic glutamine in coactivators for nuclear hormone receptors. Endocrine J 1999;46:279–84.
14. Rebbeck TR, Wang Y, Kantoff PW, Krithivas K, Neuhausen SL, Godwin AK, Daly MB, Narod SA, Brunet JS, Vesprini D, Garber JE, Lynch HT, et al. Modification of *BRCA1* and *BRCA2* associated breast cancer risk by the AIB1 genotype and reproductive history. Cancer Res 2001;61:5420–4.
15. Haiman CA, Hankinson SE, Spiegelman D, Colditz GA, Willet WC, Speizer FE, Brown M, Hunter DJ. Polymorphic repeat in AIB1 does not alter breast cancer risk. Breast Cancer Res 2000;2:378–85.
16. Antoniou AC, Phrouah PD, McMullan G, Day NE, Stratton MR Peto J, Ponder BJ, Easton DF. A comprehensive model for familial breast cancer incorporating *BRCA1*, *BRCA2* and other genes. Br J Cancer 2002;86:76–83.
17. Lange K, Weeks D, Boehnke M. Programs for pedigree analysis: MENDEL, FISHER and dGENE. Genet Epidemiol 1988;5:471–2.
18. Kadouri L, Easton DF, Edwards S, Hubert A, Kote-Jarai Z, Glaser B, Durocher F, Abeliovich D, Peretz T, Eeles RA. CAG and GGC repeat polymorphisms in the androgen receptor gene and breast cancer susceptibility in BRCA1/2 carriers and non-carriers. Br J Cancer 2001;85:36–40.
19. Easton DF, Peto J, Babiker A. Floating absolute risks—an alternative to choosing an arbitrary group in survival analysis and case-control studies. Stat Med 1991;10:1025–35.
20. Phelan CM, Rebbeck TR, Weber BL, Devilee P, Ruttledge MH, Lynch HT, Lenoir GM, Stratton MR, Easton DF, Ponder BA, Cannon-Albright L, Larsson C, et al. Ovarian cancer risk in BRCA1 carriers is modified by the HRAS1 variable number of tandem repeat (VNTR) locus. Nat Genet 1996;12:309–11.
21. Rebbeck TR, Kantoff PW, Krithivas K, Neuhausen S, Blackwood MA, Godwin AK, Daly MB, Narod SA, Garber JE, Lynch HT Weber BL, Brown M. Modification of BRCA1-associated breast cancer risk by polymorphic androgen-receptor CAG repeat. Am J Hum Genet 1999;64:1371–7.
22. Wang W, Spurdle AB, Kolachan AB, Bove B, Modan B, Ebbers SM, Suthers G, Tucker MA, Kaufman DJ, Boody MM, Tarone RE, Daly M, et al. A single nucleotide polymorphism in the 5′ untranslated region of RAD51 risk of cancer among BRCA1/2 mutation carriers. Cancer Epidemiol Biomarkers Prev 2001;10:955–60.
23. Levy-Lahad E, Lahad A, Eisenberg S, Dagan E, Paperana T, Kasinetz L, Catane R, Kaufman B, Beller U, Renbaum P, Gershoni-Baruch R. A single nucleotide polymorphism in the RAD51 gene modifies cancer risk in BRCA2 but not BRCA1 carriers. Proc Natl Acad Sci USA 2001;98:3232–6.
24. Zhuchenko O, Baily J, Bonnen P, Ashizawa T, Stockton DW, Amos C, Cobyns WB, Subramony SH, Zoghbi HY, Lee CC. Autosomal dominant cerebellar ataxia (SCA6) associated with polyglutamine expansions in the a1A-voltage-dependent calcium channel. Nat Genet 1997;15:62–9.
25. David G, Abbas N, Stevanin G, Durr A, Yvert G, Cancel G, Weber C, Imbert G, Saudou F, Antoniou E, Drabkin H, Gemmill R, et al. Cloning of the SCA7 gene reveals a highly unstable CAG expansion. Nat Genet 1997;17:65–70.
26. La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 1991;352:77–9.
27. Ingles SA, Ross RK, Yu MC, Irvine RA, La Pera G, Haile RW, Coetzee GA. Association of prostate cancer risk with genetic polymorphisms in vitamin D receptor and androgen receptor. J Natl Cancer Inst 1997;89:166–70.
28. Young IE, Kurian KM, Mackenzie MA, Kunkler IH, Cohen BB, Hooper ML, Wyllie AH. The CAG repeat within the androgen receptor gene in male breast cancer patients. J Med Genet 2000;37:139–40.
29. Chamberline N, Driver E, Miesfeld R. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acid Res 1994;22:3181–6.
30. Gerber HP, Seipel K, Georgiev O, Hofferer M, Hug M, Rusconi S, Shaffner W. Transcriptional activation modulated by homopolymorphic glutamine and proline stretches. Science 1994;263:808–11.

31. Patel MS, Cole DE, Smith JD, Hawker GA, Wong B, Trang H, Veith R, Melzer P, Rubin LA. Alleles of the estrogen receptor alpha-gene and an estrogen receptor cotranscriptional activator gene, amplified in breast cancer (AIB1) are associated with quantitative calcaneal ultrasound. J Bone Miner Res 2000;15:2231–9.
32. Jernstrom H, Chu W, Vesprini D, Tao Y, Majeed N, Deal C, Pollak M, Narod SA. Genetic factors related to racial variation in plasma levels of insulin-like growth factor-1: implications for premenopausal breast cancer risk. Mol Genet Metab 2001;72:144–54.
33. Armes JE, Venter DJ. The pathology of inherited breast cancer. Pathology 2002;34:309–14.
34. Gudas JM, Nguyen H, Li T, Cowan KH. Hormone dependent regulation of BRCA1 in human breast cancer cells. Cancer Res 1995;55: 4561–5.
35. Fan S, Wang JA, Yuan R, Ma Y, Mey Q, Erdos MR, Pestell RG, Yuan F, Auborn KJ, Goldberg ID, Rosen EM. BRCA1 inhibition of estrogen receptor signaling in transfected cells. Science 1999;284:1354–6.
36. Spillman MA, Bowcock AM. BRCA1 and BRCA2 mRNA levels are coordinately elevated in human breast cancer cells in response to estrogen. Oncogen 1996;13:1639–45.
37. Rajan JV, Wang M, Marquis ST, Chodosh LA. BRCA2 is coordinately regulated with BRCA1 during proliferation and differentiation in mammary epithelial cells. Proc Natl Acad Sci USA 1996;93: 13078–83.
38. Kauff ND, Satagopan JM, Robson ME, Scheuer L, Hensley M, Hudis CA, Ellis NA, Boyd J, Borgen PI, Barakat RR, Norton L, Offit K. Risk-reducing salpingo-oophorectomy in women with BRCA1 or BRCA2 mutation. N Engl J Med 2002;346:1609–15.
39. Rebbeck TR, Lynch HT, Neuhausen SL, Narod SA, vant'Veer L, Garber JE, Evans G, Isaacs C, Daly MB, Matloff E, Olopade OI, Weber BL. Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. N Engl J Med 2002;346:1616–22.
40. Zheng L, Annab LA, Afshari CA, Lee WH, Boyer TG. BRCA1 mediates ligand-independent transcriptional repression of the estrogen receptor. Proc Natl Acad Sci USA 2001;98:9578–92.

APPENDIX

Mutations in BRCA1/2 non-Ashkenazi carriers:

BRCA1 (n = 48): 4184delTCAA: 11 cases, 3450delCAAG: 6 cases, 3124delA: 3 cases, 3875delGTCT: 3 cases, 2 cases of each: 546G→T, 1014delT, 1445T→A, 2157delG, 2190delA, 5629delG and 1 case of the following: 122G→T, 1182A→T, 1224delGAT, 1454delAA, 1623delTTAAA, 1942delAGAA, 2012insT, |2313G>|T, 2371delTG, 2594delC, 2722G→C, 3034delAAAC, 5312delT.

BRCA2 (n = 11): 6503delTT: 4 cases, 7057delCTTAT: 2 cases and a case of the following: 1529delAAAG, 3173C→G, 6690delTC, 6819delTG, 9179C→G.